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(71) Applicant *(for all designated States except US)*: IDEA INNOVATIVE DERMALE APPLIKATIONEN GMBH [DE/DE]; Frankfurter Ring 193a, D–80807 München (DE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: CEVC, Gregor [DE/DE]; Ewrich–Kästner–Weg 16, D–85551 Kirchheim (DE).

(74) Agent: MAIWALD, Walter; Maiwald GmbH, Elisenhof, Elisenstrasse 3, D–80335 München (DE).

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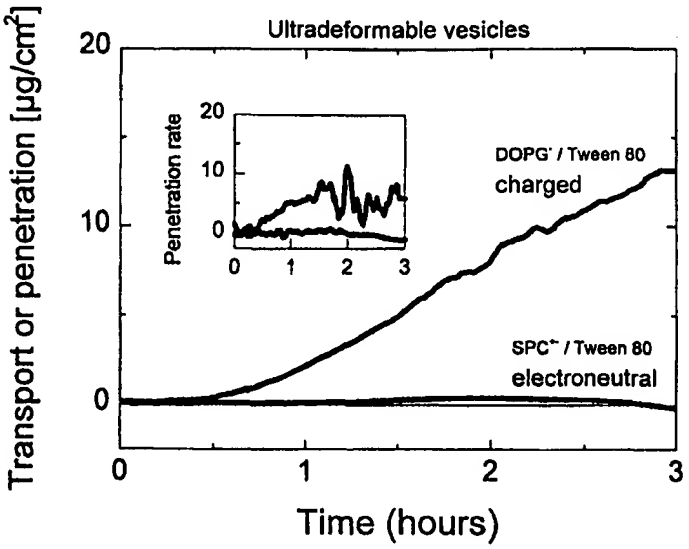
(54) Title: ELECTRICALLY CONTROLLED TRANSPORT OF CHARGED PENETRANTS ACROSS BARRIERS

(57) Abstract

It is an object of the invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and/or said agents being provided with sufficient electrical charges, to enable and/or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. It is another object of the invention to provide a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier.



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Electrically controlled transport of charged penetrants across barriers

Field of the invention

This invention relates to a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. This invention also relates to a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier.

Background of the invention

Charged entities may migrate spontaneously from the high to the low electrostatic potential site, unless prevented from doing so by an obstacle, such as a barrier. The driving electrostatic force is proportional to the total charge on an entity and to the electrostatic potential difference. Material flow also depends on the system's resistance to resulting motion. Consequently, the electrically driven transport across a barrier is sensitive to the number, width and characteristics of pores in a barrier, which together define the barrier permeability, P, and its inverse, the barrier resistance. One example

for such pourous barrier is the skin, which typically contains pores (in the unwidened state) with the diameter of a few Ångstroms, approximately.

Any transcutaneous electric potential that drives an ion flux across the skin tends to widen some hydrophilic channels in the organ. This typically happens at the worst packed sites between the cells, where the biggest opportunity for the transport enhancement resides.

Skin penetration by means of suitable carriers achieves a similar goal without the need to use gadgets or external sources of energy (Schätzlein, A.; Cevc, G. (1998): Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br. J. Dermatol. 138: 583-592). The hydrophilic passages (pores) through the skin before the treatment will only let small, e.g. water, molecules pass. Such pores can be opened into wider channels, however, by the addition of sufficiently potent penetrants.

Electrical potential difference attempts to drive charged penetrants across the barrier; for example, across the skin (*cutis*); furthermore, an electrical permeation enhancement widens at least some hydrophilic channels in the organ. This happens nearly exclusively in the horny layer of the skin (the *stratum corneum*), which contributes most to the skin permeability barrier. Depending on the size of newly opened transcutaneous pathways, it is customary to speak about electrophoresis (=iontophoresis) or electroporation, for the electrically induced flow through narrow passages and for the widening of more extended passages, respectively.

For example, an electric current of approximately 0.4 mA cm^{-2} or less will activate a small proportion of narrow (~0.5 nm) hydrophilic channels pre-existing between the cells in the skin. Such channels then remain open for many hours (Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7), but remain narrow (< 3 nm, in nude mice), when a transcutaneous voltage remains in the physiologically tolerable range (< 3 V for a 1 cm^2 patch).

The widest channels are negative inside. Neutral channels are only half as wide and the positive ones are twice smaller (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21). To date, the widest channels inferred to occur during a low-voltage electromotion through the skin were reported to be merely 20 nm in diameter or less (Aguilelle, V.; Kontturi, K.; Murtomaeki, L.; Ramirez, P. (1994) Estimation of the pore size and charge density in human cadaver skin. J. Contr. Rel. 32: 249-257).

The standard electrical skin permeability enhancement method (electrophoresis) therefor only can improve the transport of relatively small (<2 nm) charged molecules across the organ. Electrophoresisis across the skin, consequently, is feasible for certain polypeptides but is practically useless for the delivery of proteins or other large penetrants (for reviews see refs. Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7; Green, P. G.; Flanagan, M.; Shroot, B.; Guy, R. (1993) Iontophoretic drug delivery. In: Pharmaceutical Skin Penetration Enhancement (Walters, K. and Hadgraft, J., eds.) Marcel Dekker, New York, 297-319; Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. J. Pharm. Sci. 82: 240-3; Singh, S.; Singh, J. (1993) Transdermal drug delivery by passive diffusion and iontophoresis: a review. Med. Res. Rev. 13: 569-621; Singh, J.; Bhatia, K.S. (1996) Topical iontophoretic drug delivery: pathways, principles, factors, and skin irritation. Med. Res. Rev. 16: 285-96).

Transcutaneous channels opened by the low voltages and tolerably small currents only cover some 0.005 % of the total treated area, despite their seemingly high number ($< 3\times10^8$ cm^{-2}) (Pikal, M.J. (1990) Transport mechanisms in iontophoresis. I. A theoretical model for the effect of electroosmotic flow on flux enhancement in transdermal iontophoresis. Pharm. Res. 7: 118-26). More extended local skin perforations, which are created by a higher voltage (> 150 V), are rarer, but normally

persist in the skin for several days in the form of lesions.

Transcutaneous channels size is affected by a number of parameters. For example, a channel widens with increasing electrostatic potential as well as with decreasing supporting electrolyte concentration, but the latter variation is practically only possible within relatively narrow limits. Moreover, no teaching was given to date on how to improve the transport across a barrier by using such principles. Perhaps, this is due to the fact that the electrophoretic channels in the skin are charge and molecular weight selective (Banga, A.K.; Chien, Y.W. (1993) Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. Pharm. Res. 10: 697-702) but not very sensitive to the agent lipophilicity variation (Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7).

Repeated electrophoretic delivery through the same skin area results in a divergent, but typically greater, flux across the barrier which makes data interpretation and recommendations difficult (Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. J. Pharm. Sci. 82: 240-3). Further complications arise from the electrical current through the appendages in the skin, such as hair follicles.

Electrical opening of channels through the skin is reflected in the following contribution to the skin permeability,

$P_{i,el}$ = Skin Permeability to Ions = $(c_i Z_i F/RT)\, D_i/d_S$

which needs to be added to the permeability observed with no electrical force applied across the barrier. In addition to the pores opening, a transcutaneous electrical potential gradient ($\Delta\psi_{el}$) also activates the electromotive forces which try to drive charged penetrants through the channels. This gives rise to an additional term in Fick's transport

equation used to model transbarrier (e.g. transcutaneous) transport (see further discussion):

$$j_i = \ldots a\, c_i + P_{i,el}\, \Delta\psi_{el}$$

c_i is the bulk concentration of substance i, Z_iF is its molar charge (valency times Faraday constant), and RT is the molar thermal energy. D_i is the diffusivity of substance i and d_s the skin thickness. Electric current correspondng to i is given by the product of i-flux and Z_i F, but the total current comprises all individual contributions and thus is given by the sum of susch contributions.

Electrophoresis represents the direct flow of charged molecules in an electric field under the electrode. Drug molecules must therefore be placed at electrodes having a polarity of the same charge as the agent. Under such circumstances, the flux magnitude is proportional to the net number of charges on each migrating molecule and to the applied potential. Further important factors are drug concentration and diffusivity in the barrier or skin (see equation given later in the text).

Owing to absolute differences in concentrations, electrophoretic current normally also comprises contributions from the supporting electrolyte ions (e.g. Na^+, Cl^-). These are often diminant making the drug contribution only a minor part of the measured current. Increased ion concentration under the electrodes therefore lowers the useful part of electrophoretic flow (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Pikal, M.J.; Shah, S. (1990c) Transport mechanisms in iontophoresis. III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes. Pharm. Res. 7: 222-9), as can be see from simple differential calculation.

It is therefore state of the art knowledge that physical limitations restrict maximum achievable, or tolerable, electrophoretic current across the skin: currents flowing

through the already opened pores dissipate electric energy; this prevents a greater increase in the channel number and size, as stated above, and minimizes the achievable transport gain. Further pore opening is also restricted by the adverse side effects of energy dissipation in the skin (skin itching and etching).

Electro-osmotic flux , that is, the flow of water associated with the transported ions carrying uncharged species, also relies on hydrophilic channels in the skin and on the applied potential. Flux under an anode typically exceeds the cathodal values, probably due to the different average size of positively and negatively charged channels. The average flux value reaches a steady-state during 10 hours of constant-current iontophoresis (0.36 mA cm^{-2}) at the level a few times higher than at the beginning (< 3 $\mu L\ h^{-1}\ cm^{-2}$: Kim, A.; Green, P.G.; Rao, G.; Guy, R.H. (1993) Convective solvent flow across the skin during iontophoresis. Pharm. Res. 10: 1315-20).

Electrically induced changes in the skin are the greatest over the first hour of electrophoresis (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Craane van-Hinsberg, W.H.; Bax, L.; Flinterman, N.H.; Verhoef, J.; Junginger, H.E.; Bodde, H.E. (1994) Iontophoresis of a model peptide across human skin in vitro: effects of iontophoresis protocol, pH, and ionic strength on peptide flux and skin impedance. Pharm. Res. Sep; 11: 1296-300). During this period of time, the resistance drops from > 20 $k\Omega\ cm^{-2}$ to approximately 10 % of the starting value.

Skin pretreatment with ethanol reduces (Brand, R.M.; Iversen, P.L. (1996) Iontophoretic delivery of a telomeric oligonucleotide. Pharm. Res. 13: 851-4) or else increase (Srinivasan, V.; Higuchi, W.I.; Sims, S.M.; Ghanem, A.H.; Behl, C.R. (1989) Transdermal iontophoretic drug delivery: mechanistic analysis and application to polypeptide delivery. J. Pharm. Sci. 78: 370-5) the electrophoretic transport across the organ. Most chemical skin permeation enhancers improve the electroconductivity of the skin, and thus also the electrically driven transcutaneous transport (Green, P.G.; Hinz,

R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7); so does the pH adjustment, particularly lowering of pH. Part of this effect is due to electrophoresis and part to electro-osmosis, but increases are generally relatively small.

Electrophoretic enhancement of molecular motion across the skin was only partly successful to date (for a recent survey of products and developments see: Cevc, G. (1997) Drug Delivery Across the Skin. Exp. Opin. Invest. Drugs 6: 1887-1937). Particularly poor results were achieved with macromolecules (see, e.g. the reviews by Siddiqui, O.; Chien, Y. W. Nonparenteral administration of peptide and protein drugs. Crit. Rev. Therap. Drug Carrier Syst. 1987, 3: 195-208 and Banga, A.K.; Chien, Y.W. (1993) Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. Pharm. Res. 10: 697-702). For insulin, for example, the effectiveness of iontophoretic transport in the best case was 4% per hour and normally lower than 3 % per hour; part of the observed transport effectprobably being due to the skin damage (Siddiqui, O.; Chien, Y. W. Nonparenteral administration of peptide and protein drugs. Crit. Rev. Therap. Drug Carrier Syst. 1987, 3: 195-208).

This problem is partly due to the high mass, but also to the hydrophilicity of most large molecules, which both pose tremendous difficulties to the general use of conventional skin permation enhancement technology.

The situation with other large penetrants is comparably bad. To date, only one publication tackled the problem of driving large lipid aggregates, liposomes, across the skin, without finding a solution (see further discussion).

It is therefore fair to say that no procedure was known before this invention which would ensure an efficient electromotion of large penetrants across the microporous barriers, such as mammalian skin. Moreover, no generally applicable method was proposed to date for the opening of large pores in the skin. This is unfortunate in light

of the desire to deliver transcutaneously large molecules, such as peptides and proteins, but also due to the long standing desire to control aggregate motion across any kind of transport barrier.

The extent and mechanism of aggregate motion across biological barriers, such as the stratum corneum, is strongly disputed (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388), as is the penetration pathway through the skin. We have repeatedly discussed hydrotaxis as most important cause for the transport of superficially hydrophilic, highly deformable vesicles through the biological barriers, such as the skin (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388; Cevc, G. (1997) Drug Delivery Across the Skin. Exp. Opin. Invest. Drugs 6: 1887-1937). We argued that diffusion is not a good basis for transporting large aggregates, i.e. lipid vesicles, accross such barriers.

The first reason for this is the very low permeability (P_a) of any big aggregate with a large effective mass, which is typically proportional to the aggregation number (n_a). Since the P_a-value correlates with the diffusion constant (D_a), the permeability and the flux of such a large body both decrease linearly with the growing aggregate size. (P_a is thus proportional to $D_a \sim D_1/n_a$, where D_1 is the diffusion constant of a monomer.)

The second cause for the insignificance of aggregate diffusion is the smallness of largest achievable aggregate concentration difference across the barrier ($\Delta c_a = \Delta c_1 / n_a$, where c_1 is the saturated monomer concentration). As can be calculated from the first Fick's law, the flux $j_m = P_a \Delta c_a$, is hence proportional to $D_a \Delta c_a \sim D_1 \Delta c_1 / n_a^2$.

Both above mentioned phenomena, which result in $D_a(n_a >> 1) \rightarrow 0$ and $\Delta c_a (n_a >> 1) \rightarrow 0$, contribute to negligibly small barrier permeability for the vesicle transport: $P_a(n_a >> 1) \rightarrow 0$.

The use of water activity gradient (Δa_w), i.e. hydrotaxis, to drive transbarrier transport solves the first part of permeability problem. Our interpretation of this is the following: the aggregate independent water activity gradient exerts a similar attraction on all polar molecules in the aggregate; this strengthens proportionally the pressure acting on each aggregate, $\Delta p_{hyd,a} \sim \Delta a_w RT n_a$, or on the corresponding force that drives the aggregate transport across the barrier, F_{hyd}. Both are much bigger for aggregates than for a single molecule. This compensates the smallness of aggregate concentration difference, as can be seen from the generalized Fick's equation:

$$j_a = P_a \Delta c_a + P_a'' \Delta a_w RT n_a$$
$$\sim P_a' n_a F_{hyd,1}$$

$F_{hyd,1}$ denotes the force acting on each monomer in the aggregate and RT is the thermal energy.

In order to profit maximally from an 'external' transport driving force, which is (permeant/penetrant) concentration independent, one can use easily deformable aggregates described in PCT/EP91/01596. This minimizes the increase of transport resistance with increasing aggregate number, by pushing it below the suggested linear dependence (see figure 1).

One example for this are the vesicles with a membrane sufficiently flexible to result in a low vesicle deformation energy. This is especially true when the vesicles are subject to strongly anisotropic (ideally: unidirectional) stress, 'force' or pressure. The combination of molecular aggregation and membrane flexibility under the corresponding conditions, therefore, may lead to vesicle motion through a barrier even when the pores in such barrier are smaller than the vesicle diameter. Significant material flow in the desired direction can result from this.

The same consideration applies to every external, and therefore concentration

independent, force (F_{ext}) or pressure (Δp_{ext}). The proviso for this is that aggregate-size dependent increase of the force - or of the resulting trans-barrier pressure difference - exceeds the decrease of permeability (P_a) with growing aggregate size. A net trans-barrier transport results from this. The relation is schematically illustrated in figure 1. If the driving force increases linearly with the number of charges on each moving entity, and provided that the transport resistance of such entity is increases less rapidly than the driving force (full line), a net transport will ultimately result. Such is the situation with the penetrants which are adaptable in shape to the pores. When the penetrants get big enough, and the driving force exceeds the transport resistance, an effective transport sets in. If the penetrants are not adaptable to the pores in a barrier, however, the barrier resistance inevitably exceeds the driving force , when the average 'penetrant' size exceeds the average diameter of a pore (dotted line).

The above reasoning applies as long as the transport driving force is constant or as long as the increase of transport resistance does not exceed the size dependent ascent of driving force. Highly deformable aggregates subject to a sufficiently high external pressure ($\Delta p_{ext,a}$) provide an example for this; the oposite situation is encountered with conventional, less deformable aggregates under a similar pressure, since these aggregates will rather break at than pass through the barrier.

In the case of an external electrical force (F_{el}), which could lead to so-called electrophoresis, similar basic principles apply, but are more complicated and not a priori recognizable..

In the simplest, hypothetical case, in which an aggregate comprises n_a charged molecules with a charge Ze_0 each, and the corresponding counterions are the only other charged entities in the system, the rate of aggregate transport increases linearly with aggregate size or number, as well as with the electric gradient across the barrier (E). This is true as long as transport resistance remains constant, as the driving force is then given by $F_{el} = n_a Ze_0 E$, and the P_a-value is taken to be constant. However, if the transport resistance depends on aggregate-size, and also increases with the value of n_a,

the P_a-value decreases and can abolish the transport-rate sensitivity to the changes in aggregation number. When P_a-value decreases faster than linearly, since the transport resistance increase is more than linear, the transport rate decreases with increasing n_a-value even.

One would therefore only expect an efficient electrophoresis in the first of the three above mentioned cases. In reality many more problems arise. Nearly all suspensions of charged aggregates contain small ions of similar charge as aggregates, in addition to ionic aggregate components. These small, additional charges react to an electric field like aggregates and trespass the barrier in the same direction. As a consequence of this, a parasitic stream of 'small charges' begins to flow, which ultimately may dissipate the electrical potential over the barrier. If the transport resistance of such 'opportunistic charges' is smaller than that of the useful aggregates, which is usually the case, the aggregate transport can stop eventually.

A further expected complication in electrophoresis of large objects is the possibility that an applied electrical potential tends preferentially to pull individual charged molecules from an aggregate over the barrier, instead of transporting the whole aggregate. The expected size-dependence of transport resistance nourishes this notion, especially for the relatively strongly water soluble, charged aggregate components. The highly deformable aggregates, which consist of substances of different solubility (according to PCT/EP91/01596), fulfill such requirement. This rises the doubt about the suitability of the corresponding ultradeformable vesicles for electrophoresis.

The only group which published results on electrically driven material transport across the skin by using liposomes as "permeation enhancers" produced very sobering data, indeed.

In the first publication on the combined use of liposomes and iontophoresis for transdermal delivery, which should form closest prior art to the present invention, Vutla et. al. (Vutla,N.B.; Betageri, G.V.; Banga, A.K. (1996) Transdermal iontophoretic

delivery of enkephalin formulated in liposomes. J. Pharm. Sci., 85: 5-8) reported the following.

Liposomes comprised dimyristoylphosphatidylcholine /cholesterol 2/1 mol/mol mixture with an unspecified amount of cationic stearylamine or anionic phosphatidylserine, when appropriate, to make the vesicles charged. They were prepared fresh by extrusion and had a size of 110 nm. The release was much higher from neutral and negative liposomes than from the positive vesicles.

A current of 0.5 mA cm^{-2} density co-transported [Leu5]enkephalin, (spiked with [3H]enkephalin) across the skin from anode or cathode, depending on the charge on the molecule.

After 12 h of iontophoresis, liposome derived material was found in the skin at the level of approx. 2.5%, 0.75%, and 1.5% for the positive, negative and neutral vesicles, respectively; in the absence of electrical current 0.8% of material from neutral liposomes was found in the skin. No liposome derived material was recovered from receiver fluid.

The use of negatively charged vesicles did not enhance enkephalin delivery across the skin. The positive liposomes even reduced the delivery compared to control.

The polypeptide delivery into the skin was the highest (4.2%) for the neutral vesicles used in conjunction with electrophoresis (sic!), followed by the same kind of vesicles used in the absence of electrical current (passive delivery: 2.7%); anionic and cationic vesicles used with iontophoresis mediated much lower intracutaneous drug delivery of 0.5% and 0.7%, respectively.

The work of Vutla thus clearly shows that conventional liposomes, whether charged or uncharged, are poor mediators of the electrically driven material transport into the skin.

It was therefore not expected to date that electrical driving force could be used as a replacement of hydrotaxis for the purpose of transporting large lipid aggregates (e.g. vesicles) or other big entities across the skin.

In view of the foregoing it is therefore an object of the present invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores.

It is moreover an object of the present invention to provide a method for effecting the electrically driven transport of penetrants and associated molecules through the pores in a barrier by applying an electrical potential across the barrier.

These objects are attained by the invention as defined in the attached independent claims.

Further advantageous embodiments of the present invention are provided by appended subclaims.

Description of the invention

We found, unexpectedly, that charged ultradeformable lipid aggregates in vesicular form can be forced to cross artificial as well as natural nano-porous barriers with an externally applied electrical potential difference. The proviso for this is sufficient stress on the vesicles, which must be big enough to deform the vesicles. Such a situation is only realized with the vesicles with very flexible membranes. The process involving the deformation of entire aggregate, the relative magnitude of vesicle adaptation to the pore

penetration is affected by the average vesicle/pore size ratio.

We also found out that the electromotion of charged aggregates is sensitive to the bulk electrolyte concentration. Unexpectedly, the measured dependence was seen to deviate, qualitatively as well as quantitatively, from that expected on the basis of known electromotion of non-deformable, small penetrants across a barrier. The electrically driven motion of the highly deformable vesicles across "confining" pores therefore differs from conventional electrophoresis and provides new means for the delivery of drugs across various, including biological, barriers.

It stands to reason that nonocclusive pretreatment of the skin with a suspension of ultradeformable aggregates - and subsequent application of transcutaneous electric potential - can increase the final electrophoretic flux. We speculate that this might be due to the opening of channels in the skin by non-electrical means. Such an increase in overal penetrability of the organ, which relies on more efficient, non-electrostatic channels opening, can be exploited subsequently to deliver loaded charged vesicles across the barrier. The latter are then pushed through pretreated skin by the transcutaneous electric potential applied under occlusion.

Last but not least, it is also plausible to postulate that, contrary to previous belief, large molecules can be delivered efficiently across the skin. Their delivery is made possible after macromolecular association with the charged ultradeformable carriers and involves transcutaneous electromotion of such unusually adaptable, protein-carrying transporters.

We further discuss some relevant properties of molecular aggregates/associates suitable for the use in conjunction with electrophoresis. We concentrate only on the complex bodies that can overcome transport barriers under the influence of a transbarrier electrical gradient of sufficient magnitude. We describe the basic experiments relevant for this phenomenon and interpret their results. We propose general conclusions useful for the application of concepts advocated in this work in the widest possible sense. Particularly interesting, but not exclusive, is the use of our novel approach in the human

and veterinary medicine.

In the present invention, permeation denotes diffusive, concentration driven motion of molecules across a barrier.Penetration describes the non-diffusive motion of large penetrants across a barrier; the process typically being associated with a penetration induced decrease in the barrier resistance (pore widening or channel opening).

A penetrant, consequently, is any entity comprising a single molecule or an arrangements of molecules too big to permeate through the barrier. A permeant, on the other hand, is an entity that can permeate through the (semi-permeable) barrier. A penetrant in an external field experiences driving force proportional to the nominal penetrant size and to the applied field. Such a force may push the penetrant through the barrier, such as the skin, if the force is strong enough either to deform the penetrant or else to widen the passages in the barrier sufficiently to elude the problem of size exclusion, or both. In the skin, for example, a transport-driving force must first intercalate the penetrant between cells to form channel wider than the effective penetrant size. (To achieve a high rate of penetrant transport, the effective penetrant size should be much smaller than the nominal penetrant size.) This goal is best achieved by the penetrants that are controllably and stress-dependently deformable. The average diameter, the electrical charge and/or the adaptability in shape or size to the pores of the penetrant is selected so as to enable electromotion.

Electrical potential gradient (across a barrier) means an arbitrary potential difference of any sign or magnitude, unless otherwise specified. Specifically, it is not necessary to place the potential generating electrodes directly on the barrier; any placement resulting in trans-barrier gradient is acceptable. "Potential difference" is used as a synonym for "potential gradient".

For further definitions, especially such pertaining to the highly deformable complex bodies (aggregates) and their mechanism of action, as well as for the list of selected interesting agents, we explicitly refer to our issued or pending patents (DE 41 07 152,

PCT/EP91/01596, PCT/EP96/04526, DE 44 47 287). The same patents also contain detailed descriptions of the essential properties and characteristics of such aggregates.

In short, lipid aggregates should be able to compensate their deformation-induced, local stress (deformation energy) in order to be extremely deformable. This can be accomplished by adjusting their local composition to such a stress, which is only possible if aggregates comprise at least two components. The carrier ingredients are conventiently chosen so that the component which can sustain the deformation better is accumulated while the less adaptable component is diluted at the maximally stressed site. This results in a transient instability (metastability) which must be sufficiently short-lived not to compromise the aggregate integrity. Highly deformable vesicles named Transfersomes in the above mentioned patents (applications) were designed specifically to meet this need and to comply with the requirements for aggregate ultradeformability.

Preparation temperature is normally chosen in the 0 to 95 °C range. Preferrably, one works in the temperature range 18-70 °C, most frequently at temperatures between 15 and 45 °C. On the skin, 32 °C are normally measured. Other temperature ranges are possible, however, most notably for the systems containing freezable or non-volatile components, cryo- or heat-stabilizers, etc.

If required to maintain the integrity and the desired properties of individual system components, carrier formulations can be stored in cold (e.g. at 4°C), with or without an associated active. Manufacturing and storage under an inert atmosphere, e.g. under nitrogen, is also possible and sometimes sensible. The shelf-life of (drug-loaded) carrier formulation, moreover, can be extended by using little unsaturated substances, by the addition of antioxidants, chellators, and other stabilizing agents, or by the ad hoc preparation from a freeze dried or dry mixture.

In the majority of cases the application is done at ambient temperature. An administration of useful suspension and potential application at lower or higher

temperatures are also possible. They make particular sense with the formulations comprising from synthetic subtances which are rigid between the room and skin or other barrier temperature.

Formulations for the use in conjunction with electrophoresis can be processed at the site of application. For lipid vesicles, both charged and uncharged, examples are given in our previous german patent application and in the handbook on `Liposomes' (Gregoriadis, G., Hrsg., CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography `Liposomes as drug carriers' (Gregoriadis, G., Hrsg., John Wiley & Sons, New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If required, any suspension of drugs can also be diluted or concentrated (e.g. by ultracentrifugation or ultrafiltration) just before application; additives can also be given into a formulation at this time or before. After any system manipulation, the carrier characteristics should be checked and, if required, readjusted.

This invention concerns a preparation comprising penetrants formed by single molecules or by arrangements of molecules, in which said penetrants are capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores. In this the penetrants are capable of transporting agents through the pores in the barrier. When the penetrants enter the pores a pore widening and channel opening by the penetrants results. Therefore, alternatively, agent permeation through the (now opened or widened) pores in the barrier is enabled subsequently to penetrant entry into said pores. The average diameter and the adaptability of said penetrants are selected, and said penetrants and / or said agents are provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or in the alternative case agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability

According to the invention it is preferred if said penetrant is provided with sufficient

electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being selected to achieve, and in case, control said transport through the barrier under the influence of the electrical driving force.

It further is preferred if said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force

Furthermore it is preferred if said penetrant is capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their permeation through the pores of the barrier subsequent to entry of said penetrant into said pores.

Said electrically charged penetrants or the charged association of penetrant and agent have preferably an average diameter which is greater (by at least the factor of 2) than the average diameter of the pores of the barrier.

In a particular embodiment of the invention a preparation is described which is characterized by the fact that the electrically charged penetrant is formed by an electrically charged single molecule or an arrangement of electrically charged molecules and is associated with one or several charged or uncharged agent molecules.

As a variation the above mentioned penetrant is formed by an electrically neutral single molecule or an arrangement of electrically neutral molecules and is associated with at

least one electrically charged agent, the quantity of electrical charges being sufficient to enable transport.

In a preferred embodiment of the invention said penetrants are suspended or dispersed in a liquid medium and comprise arrangements of molecules in the form of minute fluid droplets surrounded by a membrane-like coating of one or several layers of at least two kinds or forms of amphiphilic substances with a tendency to aggregate, said at least two substances differing by at least a factor of 10 in solubility in the, preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or the average diameter of hetero-aggregates comprising both said substances is smaller than the average diameter of homo-aggregates of the less soluble substance.

It turns out to be advantageous if the more soluble substance is the agent to be transported through the barrier, and has a propensity to form common larger structures with the less soluble substance. The common structure may comprise a physical or chemical complex of the substances.

According to the invention it is convenient if the more soluble substance tends to solubilize the penetrant droplet and the content of this substance is up to 99 mol% of the concentration required to solubilize the droplet, or else corresponds to up to 99mol% of the saturating concentration in the unsolubilized droplet, whichever is higher.

It is preferred if he content of the more soluble substance is below 50 %, especially below 40 % and most preferably below 30 %, of the respective solubilizing concentration of said substance.

According to the invention the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.

It is advantageous if the less soluble self-aggregating substance is a lipid-like substance

and the more soluble substance is a surfactant.

It is preferred if the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.

It is also preferred if the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.

According to the invention it is further advantageous if the average net surface charge density on a droplet is between 0.05 Cb m^{-2} (Coulomb per square meter) and 0.5 Cb m^{-2}, preferably between 0.075 Cb m^{-2} and 0.4 Cb m^{-2}, and particularly preferably between 0.10 Cb m^{-2} and 0.35 Cb m^{-2}.

It is preferred if the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

It is also preferred if the weight amount of droplets in formulations for the use on human or animal mucosa is 0,0001 to 30 weight-%.

Specific embodiments of the invention are disclosed in which the agent is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and/or bronchospasmolyticum, an antibioticum, antidrepressivum and/or antipsychoticum, an antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an antihistaminicum, antihypertonicum, an antihypotonicum,

anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardiotonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antiflue agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance such as an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter (agonist) fot the activity of any of the above-mentioned agents or any combination of such agents.

It is particularly advantageous if the liquid medium characteristics, especially the concentration and the composition of the supporting electrolyte, are selected so as to enable and / or control the rate or the efficiency of transport of the penetrant through the pores of the barrier.

According to the invention the supporting electrolyte, in particular a buffer, is selected among monovalent (1:1) or other low valency electrolytes, with the bulk concentration preferably below 150 mM, more preferably below 100 mM, even more preferably below 50 mM, and particularly preferably up to 10 mM.

Further, according to the invention a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier, as above defined, is provided which is characterized by the fact that a sufficient electrical potential is applied across the barrier.

According to the invention the electrodes used to generate the electrical potential across the barrier are located on opposite sides or on the same side of the barrier and are arranged so as to ensure that most of the resulting electrical current will flow across the barrier.

It is preferred if the applied electrical potential value is chosen to be below 30 V, more often below 15 V, and even more preferably below 10 V, per cm^2 of the barrier surface. It is advantageous if the current driven across the barrier by the applied electrical potential is in the physiologically tolerable range, typically below 2 mA cm^{-2}, preferably below 1 mA cm^{-2}, more preferably below 0.6 mA cm^{-2} and most preferably up to 0.4 mA cm^{-2}.

Further it is preferred if the electrode size is less than 200 cm^2, more preferably below 100 cm^2, especially below 50 cm^2, most preferably below 10 cm^2, or even below 5 cm^2.

According to the invention the electrically conductive material on or of the electrodes comprises at least one metal, in particular selected from precious metals, such as silver or palladium, and/or biocompatible salts or chemical complexes of such metals, preferably the biocompatible chlorides, and most preferably silver chloride.

It is advantageous if at least one the electrode compartment is loaded with electrically charged penetrants.

It has also been shown that it is advantageous if the electrode is loaded at the application site or earlier.

It then is preferred if the electrode is loaded shortly before application, preferably within 360 min, more preferably within 60 min and even more preferably within 30 min.

In a preferred embodiment of the invention the electrode is loaded with the electrically charged penetrant pre-associated with molecules to be transported, in particular

(biologically active) agents.

In another preferred embodiment of the invention the electrode is loaded with the penetrant and the molecules to be transported, in particular agents, that associate therewith during or after said loading.

In preferred embodiments of the invention one or more programmable, preferably small, hand-held or self-supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied electric potential.

It is advantageous if different treatment areas are selected to control the transport.

In another preferred embodiment of the invention the barrier is pretreated before initiating he electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

It is preferred if the charged or uncharged penetrants used to pre-treat the barrier are similar or identical with those employed for the subsequent electrically driven transport.

It is advantagous if the charged or uncharged penetrants are non-occlusively applied for up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to the initiation of electrically driven transport of charged penetrants and/or permeants across the barrier. It shall be emphasized that it is a characteristic feature of the present invention that the electrically driven transport of permeants, i.e. any entity being capable to permeate through the pores in the barrier, may be enhanced by a pre-treatment of the barrier as above described before initiating the electrically driven transport of the

permeant.

It further is advantageous if the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

Hereinafter, several illustrative examples of the invention's systems and methods are given; it will be understood that these neither define nor imply limits of this invention. All temperatures are in degree Celsius, carrier sizes are in nanometers, ratios and percentages are given in molar units, unless stated othwerwise. Standard SI units are used otherwise in the text.

EXAMPLES

General experimental setup and sample preparation

Highly adaptable charged aggregates in the majority of cases studied in this work comprised anionic dioleoylphosphatidylglycerol (DOPG). Additional lipids with detergent or surfactant-like properties (typically the non-ionic Tween 80) were incorporated into lipid bilayers to increase the membrane flexibility. Increasing surfactant-to-lipid ratio made the vesicular aggregates more and more deformable, up to the concentration at which membrane stability was negatively affected by the detergent.

The total lipid concentration was typically 5 w-% and typically diluted to 0.5 %, unless stated otherwise. The bulk phase included buffering ingredients (10 mM) as well as, in some cases, dilute electrolyte (NaCl).

Laboratory made platinum electrodes were used in commercial glass-holders (Crown

Glass, Inc, USA), fixed to the device with a metal clamp. Hard-plastic covers provided with two openings for filling and sampling were pressed tight (sealed with O-rings) on the holder. During experiments, one of these openings was always open to let gas produced by water hydrolysis escape. This should, and has, prevented the bursting of the membrane/holder arrangement.

Freshly cleaned electrodes were separated from the receiving fluid with a microporous membrane (10 nm on the blank side and, for example, 30 nm on the test side). On the donor side, the filling volume was substantially bigger (1.2 mL) than on the blank side (14.5 mL), where the electrode was kept as close to the barrier as possible. The holder was used in a horizontal position to permit stirring of the receiver fluid. Stirring was achieved with a small magnetic bar that revolved on top of the tested barrier. The microporous barrier served as a surrogate or "artificial" skin, for the purposes of this study.

Electrical boundary conditions were defined and maintained with a constant current source (Phoresor: Iomed, Salt Lake City, USA; typical error: 0.1 mA) or a constant voltage source (Siemens, Munich, Germany; typical error: 1 mV). The test suspension was in contact with the cathode, whereas the blank sample volume was contacted by the anode.

The receiving fluid contained charged polymers (alginic acid: 0,25 w-%). These buffering polymers were first dissolved in salt-free water from an Elgastat purification unit (ELGA, UK) and then adjusted to the desired pH range (between 7 and 7.3) by titration with 0.01 N sodium hydroxide. To avoid changes in the mixed lipid vesicles composition, the fluid in the receiver compartment also contained 10^{-5} M of the most soluble vesicle component, that is, the critical micelle concentration of Tween 80. Benzyl alcohol (0.5 volume %) was added to prevent microbial system contamination during the experiments. The receiver fluid was forced by a peristaltic pump to circulate through the cuvette (placed in a fluorimeter) and to pass through the sampling cell into which a pH electrode was inserted. All experiments reported here were run at 37 degrees

Celsius.

Readings were taken continuously. The data were transformed into an electronically analysable and storable file using a XT-IBM micro-computer, equipped with an AD-converter and our own dedicated software.

For the data comparison we focused on the starting period, during which the electrical boundary conditions changed by a few percent only. The changes were estimated as good as possible and used to assign the errors shown in some figures.

To avoid false positive results, the completeness of label-aggregate association was confirmed early during the experimental work. To determine the relative amount of surfactant-solubilized label in the small mixed lipid micelles or in other kind of complexes, various suspensions were tested. This was done by pushing suspension through the membranes with 10 nm pores. Resulting fluxes were typically very small for the DPH labelled suspension. The corresponding flux values, nevertheless, were subtracted from the final flux of vesicles (that do not cross 10 nm pores). In experiments with epidermis, Rho-DHPE was used as the fluorescent label. Rho-DHPE is still highly lipophilic, but more soluble than DPH. Background signals with the former label were therefore higher than in the case of DPH-labelled vesicles and are shown in the figures directly rather than after subtraction from the other data.

Examples 1-2:

Aggregate charge effect

Uncharged highly deformable vesicles:

274 mg phosphatidylcholine (SPC)

226 mg Tween 80 (Tw80)

0.1 mol-% DPH (relative to SPC)

99.5 mL phosphate buffer, 10 mM, pH 7 - 7.3

Vesicle/pore size ratio: 3.3

Charged highly deformable vesicles:

274 mg phosphatidylglycerol ($DOPG^-$, as above)

226 mg Tween 80

0.1 mol-% DPH (relative to SPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.7

Electrical current: 1.2 mA (current density: 0.279 mA/cm^2)

Preparation of test suspension. The lipid mixture was suspended in dilute electrolyte. A sterile glas container containing crude lipid suspension was covered tightly and stirred magnetically for 3 days at room temperature. To narrow down the vesicle size distribution, the suspension was sequentially extruded through polycarbonate membranes of Nucleopore type with a nominal pore size of 400 nm, 100 nm and 50 nm, respectively. This was done at least 20 times. Vesicle suspension was then frozen and thawed 5 times at -70°C and + 50°C, respectively. To get the desired final vesicle size, suspension was re-extruded, 4 times through a 100 nm filter at 0.7 MPa. Finally, the suspension of highly deformable vesicles was sterilized by filtration through a sterile filter with 200 nm pores (Millipore) and stored at 4 °C.

Electrophoretic measurements. First, the background diffusion of label molecules was determined. This was done for several hours without applying an electrostatic potential. Next, constant electric current was set and maintained across the barrier. During this second period, pH in various parts of the test system was monitored. In receiver compartment a digital pH meter was used whereas in donor compartment dip-sticks were employed. Electrical potential difference across the barrier was permanently assessed and recorded as well. Concurrently, the electrical barrier resistance was calculated (from the measured potential and current data using Ohm's law).

Fluorescence increase in the flow-through cuvette was monitored continuously. Fluorescence increase was identified with the transported amount of material. This was done by using results from separate calibration measurements, during which known amounts of labelled suspensions were added directly into the receiving compartment. The flow of lipophilic fluorescent label (DPH) across the barrier is believed to be representative of the electrically driven vesicles motion through the barrier. The transport data given in figure 1, consequently, correspond to cummulative effect of vesicle penetration through the barrier. The measured data reveal dramatic differences in the transport of charged and uncharged mixed lipid vesicles through "confining" pores in the barrier. This clearly demonstrates the electromotive nature of aggregate transfer discovered and explored in this work.

The lack of noncharged vesicle transport notwithstanding, the applied transbarrier potential does drive a flux of small charged molecules (chiefly ions) across the barrier, as seen from the maintainance of constant current condition.

Figure 2: Time dependence of material and vesicle transport across a barrier with an applied electrical potential difference of 1.2 V, which gives rise to the trans-barrier electrical current of 0,279 mA cm^{-2}. Charged and uncharged, zwitterionic, lipid vesicles were tested.

Examples 3-4:

Aggregate deformability effect

Conventional charged vesicles, liposomes:

500 mg phosphatidylglycerol (DOPG)
(prepared from from soy-bean phosphatidylcholine)
0.1 mol-% DPH (relative to DOPG)
99.5 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 2.9

Highly deformable charged vesicles:

274 mg phosphatidylglycerol (DOPG, as above)

226 mg Tween 80

0.1 mol-% DPH

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical current: 1.6 mA (current density: 0.381 mA cm^{-2})

Results obtained with conventional vesicles differ completely from the data measured with highly deformable vesicles: simple charged liposomes do not cross 30 nm pores in the barrier under the influence of an electrical (or, in fact, any other) driving force. The fact that no significant motion of the labelled molecules across the barrier is detected for at least 6 hours supports this conclusion. Conversely, the vesicles with a highly flexible and deformable, and thus better adaptable, membrane tend to move through the narrow pores in a barrier, when they are driven in the right direction by sufficiently strong transbarrier electrical potential difference.

Based on the fact that common lipid vesicles (liposomes) only cross the pores that are bigger than their own diameter, one would expect negligeable aggregate penetration through the openings much smaller than the average vesicle diameter. Figure 1 contains unexpected and unprecedented data that put this expectation in question and require new concepts for explanation.

Results shown in figures 1 and 2 can be interpreted, for example, by generalizing the model of ultradeformable aggregate penetration described by the applicant (see e.g. Crit. Rev. Therapeutic Carrier Syst., 1997). The basic considerations for making such model modification are given in the introductory part of this application.

Figure 3: Vesicle transport (penetration) across a microporous barrier, deduced from the delivery of vesicle-associated DPH fluorescence, as function of time. Data suggests that liposomes that are ~3 times bigger than the pores cannot pass these obstacles, in contrast to the comparabaly large, but much more deformable, mixed lipid vesicles with composition that renders their membranes more flexible.

Examples 5-10:

Effects of vesicle size and electrical potential difference across the barrier

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

0.1 mol-% DPH (relative to DOPG)

226 mg Tween 80

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 5.2

Electrical driving force or parameters.

Current: 1.8 mA, 2.3 mA, 2.5 mA, 2.8 mA, 3 mA, 4 mA

Current: density: 0.429 mA/cm^2, 0.547 mA/cm^2, 0.595 mA/cm^2, 0.666 mA/cm^2, 0.714 mA/cm^2, 0.952 mA/cm^2

Experimental procedures were as described in examples 1-4. However, in this test series the effect of electrical potential difference was studied. This was first done by using relatively large vesicles which exceeded the average pore size by more than the factor of 5.

The results of this experimental series shown in figure 3 document the necessity of

applying at least 1 V potential difference acros the barrier. 1.1 V to 1.2 V are sufficient to ensure significant transport of the highly deformable vesicles through 30 nm pores. To transport greater material amount through the barrier, trans-barrier potential in excess of 1.5 V is needed. Electrostatic potential differences of such magnitude then results in rather high (opportunistic) electrical currents greater than 0.5 mA cm^{-2}.

It is therefore obvious that electro-passage of highly deformable vesicles through a barrier differs qualitatively from the simple electrophoresis or vesicle transport in the bulk. From Ohm's law one would predict that the electrically driven current will increase linearly with the transport driving potential, commensurate to the system conductivity / inverse resistance. Such a linear dependence, and constant resistance, is indeed observed during the conventional electrophoresis. In this study, however, a strong nonlinearity was found. This cannot be a consequence of changing barrier properties. The data displayed in figure 3 thus suggest that vesicle capability to cross a barrier increases with the applied potential. Similar report was made previously for the hydration-driven transport of highly deformable vesicles across a microporous barrier, and was explained with the mechanosensitivity of highly deformable mixed lipid membranes.

Figure 4: Temporal characteristics (upper panel) and potential sensitivity (lower panel) of the vesicles with an aggregate/pore size ratio of approximately 5.2, penetrating the transport barrier under influence of an external, transport driving electrical potential.

Examples 11-16:

Suspension characteristics:

As in examples 5-10, except for a decrease in

Vesicle/pore size ratio: 4.6

Electrical driving force or parameters.

Current: 1.4 mA, 1.6 mA, 1.8 mA, 2 mA, 2.5 mA, 3 mA

Current: density: 0.333 mA/cm^2, 0.381 mA/cm^2, 0.429 mA/cm^2, 0.476 mA/cm^2, 0.595 mA/cm^2, 0.714 mA/cm^2

Experiments were done and analyzed as discussed in examples 1-11. Notable difference was the decreased relative vesicle size, however, which shifted minimum potential difference required for a substantial vesicle penetration across the barrier to 1.2 V (see figure 4). Even with the highest potential difference studied to date (1.7 V), no clear proof of the saturation of potential-dependent transport increase was obtained. Significant, albeit smaller fluxes were measured with the electrostatic potential differences above 0.8 V, however.

Figure 5: Characteristic time course (upper panel) and potential sensitivity (lower panel) of ultradeformable vesicle penetrating through the pores nearly 4.6 narrower than the average aggregate diameter.

Examples 17-28:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical driving force or parameters.

Current: 0.4 mA, 0.6 mA, 0.8 mA, 1.2 mA,

1.4 mA, 1.5 mA, 1.6 mA, 2.3 mA, 3 mA,

3.5 mA, 4 mA

Current: density: 0.095 mA/cm^2, 0.143 mA/cm^2, 0.190 mA/cm^2, 0.286 mA/cm^2, 0.333 mA/cm^2, 0.357 mA/cm^2, 0.381 mA/cm^2, 0.547 mA/cm^2, 0.714 mA/cm^2, 0.833 mA/cm^2, 0.952 mA/cm^2

Results. Proper pore penetration is observed when the transbarrier electrostatic potential difference is at least 1 V. Significant, albeit smaller fluxes are measured when the difference exceeds 0.8 V.

Transbarrier potential difference bigger than approximately 1.3 V appears to make the flux of DPH (and by inferrence, the transport of vesicles) less sensitive to changes in the electrical transbarrier driving potential. It is not entirely clear whether or not the diminished increase in penetration capability, measured with the highest explored potential difference, is diagnostic of saturation of the potential dependent changes in the vesicle transport (see examples 29-35), or else is simply due to the experimental irreproducibility. Data given in the middle panel of figure 5 circumstantially support the former interpretion: if the transport is not analyzed as a function of time but rather as a function of time required to bring certain number of non-confined ions across the barrier, all the curves measured with driving potentials higher than 1 V group closer together.

Figure 6: Effect of electrostatic potential difference on the transport of highly deformable, intermediate size vesicles passing 30 nm pores. Upper panel: time course of flux measured under the constant current conditions; middle panel: data as above, but with the time-axis normalized with relatively to the given electrical current; lower panel: capability of ultradeformable vesicles to penetrate pores of fixed-size by electromotion.

Examples 29-35:

Suspension characteristics:

As in examples 17-28, except for

Vesicle/pore size ratio: 2.6

Electrical driving force or parameters.

Current: 0.25 mA, 0.4 mA, 1 mA, 1.2 mA, 1.4 mA,

1.8 mA, 2.3 mA

Current: density: 0.060 mA/cm^2, 0.095 mA/cm^2,

0.238 mA/cm^2, 0.286 mA/cm^2,

0.333 mA/cm^2, 0.429 mA/cm^2, 0.548 mA/cm^2

Test conditions in this experimental series were such that the exclusion criterium for the lipid vesicles motion across a barrier with the vesicle/pore size ratio of 2.6 was very weak. (It is known from previously published work by us (Cevc et al., Biochim. Biophys. Acta 1368, 201-215, 1998) and the others that size exclusion begins to govern the transport across microporous barriers when the penetrant/pore size ratio exceds the value of 2. The flux of vesicle-associated label, consequently, was biphasic in this test series (cf. figure 6). Normalization of the time axis (see the middle panel of figures 5 and 6) does not group the curves together. Rather than this it makes the spread more uniform.

Initial slope of material transport curve measured for different transbarrier potential and current values is fairly constant. This is illustrated in lowest panel, which gives normalized slopes of the curves shown in upper panel. The "early part" of measured curves reveals little, if any, voltage dependence. In contrast, the later time flux characteristics (after approx. 1 hour) are indicative of a change in the system properties, which is seen when transbarrier voltage or current is sufficiently high (0.7 V and 0.225 mA cm^{-2}, respectively). The observed lag-time is fairly insensitive to the electrical current, as can be seen from the upper panel of figure 2, but does get somewhat shorter with increase in current/potential value.

Barrier penetrability can not increase significantly upon changing the applied voltage. It

is therefore more than probable that the above mentioned late penetrability change results from increased capability of lipid aggregates to pass the barrier. We interpret this difference as a sign of a moderately increased vesicle adaptability to pore narrowness. The earlier transbarrier transport, on the other hand, is likely to be due to simple electrophoresis of relatively tiny vesicles. Obviously, many such vesicles are small enough to cross the pores in a barrier, probably in the process of an electrically mediated (or supported) "diffusion".

Penetration capability data illustrated in the lower panel of figure 6 are diagnostic of complete vesicle adaptability (maximum membrane flexibility), as can be seen from the fact that several high potential values are nearly the same.

Figure 7:Elektromotion of relatively small, highly deformable vesicles through 30 nm pores in a barrier. Upper panel: absolute ponetration of vesicles, as calculated from the measured DPH flux; middle panel: the same data as above, as a function of normalized time; lower panel: relative penetration capability of tested system (=DPH-derived vesicle flux per unit potential). Two different transport rates (ϕ1 and ϕ2) are seen, indicative of two different unerlaying transport phenomena.

Examples 36-40:

Effects of electrolyte concentration

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

Buffer as in previous examples

NaCl concentration (final): 1 mM, 10 mM, 20 mM, 50 mM, 100 mM

Vesicle/pore size ratio: 3.3

Electrical current: 1.2 mA; current density 0.286 mA cm^{-2}

In this test series we have shown that increasing supporting electrolyte concentration strongly affects the efficiency of electrically driven transport across a microporous barrier. High electrolyte concentrations typically support the salt transport but lower the transbarrier flux of aggregates. Above certain treshold concentration, which is believed to depend on the barrier as well as penetrant properties, the added salt may bring the transport of large aggregates to a halt.

Comparison of material flux, ion current and driving electrical potential data measured in this and previous set of experiments provides a clue to explaining the salt-dependent suppression of aggregate electromotion through the narrow pores. The relative contribution of small anions (here Cl^-) flow across the barrier is proportional to the bulk salt concentration. A lower driving potential, consequently, suffices to maintain a constant current across the barrier at higher salt concentrations (see lower panels in figure 7). Lower driving potential simultaneously lessens the ease of, and thus the probability for, large penetrant adaptability and penetration capability (see upper right panel). The latter is the proviso for an efficient flow of aggregates, however, through the narrow pores. Below certain adaptability value, which is affected by the penetrant/pore size ratio, the deformability of aggregates therefore becomes so low that only insignificant transport takes place.

Addition of salt to the suspension of complex aggregates capable of very strong, stress driven deformation therefore detrimentally affects the transbarrier transport.

Figure 8: Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores in the presence of different salt solutions. Upper left: transbarrier flow of DPH labelled vesicles; upper right: penetration capability of complex aggregates; lower left: electrical potential

that drives the constant current across a barrier as a function of time; lower right: transport driving electrostatic potential as function of bulk NaCl concentration.

Examples 41-50:

Changes in the barrier properties and in the test system characteristics

Suspension characteristics:

As in examples 17-28

Electrical driving force or parameters.

As in examples 17-28

Figure 8 shows that the electrical resistance to electrophoretic motion across a barrier increases nearly linearly with the applied electrical potential, but only in certain range. The onset of material flux, in parallel, gets faster (see figure 5 for comparison). This means that the lag-time becomes shorter with increasing transbarrier potential difference. Below the "linear" range, which commences at approximately 1 V for the tested suspension, only insignificant vesicle transport is observed. The tiny flow of aggregate material is then hardly affected by the applied potential or by the changing electrical current.

During experiments done in this test series, pH in the receiver compartment dropped by approximately 1.5 to 1.8 units, nearly independent of the applied voltage. Over the first hour of electrophoresis the change was smaller than 1 unit. (Such a variation was considered to have had only a small, if any, effect on the vesicle electromotion.) In parallel, the donor compartment pH became more alkaline by the corresponding amount. This latter variation did not change the charge on the mixed lipid vesicles, owing to the low pK= 2.9 of ionic PG in the mebranes. Lipid degradation, which is faster when the charged membranes diverge from their optimum at pH ~7.1, is believed

to have been insignificant during the first part of experiment at least.

Figure 9: Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores. Upper left: barrier electrical resistance; upper right: electrical potential required to drive constant electrical current across the barrier; lower left: pH value in the receiver compartment containing alginic acid; lower right: pH of suspension of highly flexible vesicles present in the donor compartment.

Data interpretation. When the rate of vesicle transport across a barrier is substantial, the electrical potential difference that needs to be applied to drive a constant electrical current through the pores first rapidly decreases and finally increases with time. We believe that the former phenomenon results from the redestribution of highly mobile ions in the system, especially in front of the electrodes and near the barrier. The secondary, and much slower increase, in our opinion, is largely due to the gradual pore clogging by the large or poorly deformable vesicles accumulated in front of the barrier.

Changes on or near the electrodes could partly explain the secondary changes in barrier penetrability / driving potential values. Especially with the high currents we often saw material (alginic acid?) precipitation near the reference electrode. Electrode surface also always turned brown during the course of an experiment; the higher was the applied potential or the bigger was the resulting current the more this was the case. Last but not least, hydrogen and oxygen evolution in the solution, which occasionally led to slight suspension foaming, also could have contributed to the above mentioned barrier resistance changes.

Examples 51-53:

<u>Transport characteristics of the skin (epidermis)</u>

Electrical parameters: as shown in figure 9

(Currents are given in the panels)

Electromotion through the epidermis in vitro can be used to study someof the characteristics of electrophoresis in vivo. The proviso for this is the use of sufficiently large and intact skin segments with a functional barrier. In order to obtain at least semi-quantitatively reliable data, such skin piecee must also be as thin as possible. Ideally, one would like to work with a mere barrier, that is, with the stratum cornuem only. In practice it is impossible to achieve this task, owing to the fragility of the horny skin layer. The
best that one can do then is to prepare thin but sufficiently extended pieces of the epidermis.

Electrical resistance of the epidermis is a good marker for the skin intactness. It is also diagnostic of any major changes in the barrier properties of the organ.

An example for the variable electrical resistance of the skin as a function of time during transepidermal electriophoresis is given in figure 9.

Figure 10:Electrical resistance of epidermis during serial electrophoresis experiments done in vitro.

The specific resistance of excised skin (originally somewhat higher than 10 kOhm cm^{-2}) always decreases with the cummulative current that has flown through the skin to approximately 10-20 % of starting value. This observation as well as the starting specific resistance value is comparable to the published information, which gives specific resistance values as below 20 kOhm cm^{-2} for human and murine skin. The somewhat higher resistance decrease to approximately 10% of starting value could be due to the difference in total, cummulative current.

We observed no significant difference in electrical resistance, or its time and current variation, between the tested human and porcine skin samples.

Examples 54-57:

Barrier (epidermis) thickness effect:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

Buffer as in previous examples

Part A: Vesicle/pore size ratio: 3.3;

electrical current: 0 mA, 1.2 mA (current density 0.286 mA cm^{-2})

electrical potential: difference: 0 V, 2,0 V

Part B: Vesicle/pore size ratio: 2.8

electrical current: 1.2 mA (current density 0.286 mA cm^{-2})

electrical potential: difference: 3.7 V, 5.4 V, and 7 V

Results. In first experiment (part A), an electrical current of approximately 0.3 mA cm^{-2} was shown to co-transport anly a small amount of fluorescently labelled ultradeformable vesicles through the thick epidermis, prepared by heat-separation and 2 hours of trypsin action; only approximately 6 micrograms of material have passed through each square centimeter in approx. 4 hours. Then, a collapse in the skin barrier resulted in strong decrease of electrical resistance of the skin and in a concomitant increase of material flow through the (probable) perforated organ.

Further experiments (part B) were done with two skin preparation methods. 2 hours and 7 hours of enzymatic action were used for this purpose, which gave rise to rather thick (5,4 V; 3,7 V) or thin (7 V) specimen, respectively. Moreover, slightly smaller vesicles were used than in part A. This latter difference notwithstanding, the results from repeat experiments have confirmed the trend observed in part A experiment. They also revealed the importance of skin thickness on the effective vesicle flux across the barrier.

After a lag-time of approximately 22 min the transport of ultradeformable vesicles across thin skin, as assessed by means of fluorescent label flux determination in part B, was substantial (0.4 microgramms cm^{-2} min^{-1} or approx. 25 microgramms cm^{-2} h^{-1}, see figure 11). Conversely, an order of magnitude smaller flux was measured with the two thicker epidermis samples. However, even in the case of high flux, saturation was observed. This could result from the clogging of pores in the skin, which are available in limited number, especially under conditions as used in this test.

Figure 11: **A)** Electrically driven transport of ultradeformable vesicles across human epidermis (upper panel), electrical resistance of the barrier (middle panel) and pH in receiver compartment (lower panel) during an electrophoretic experiment.
B) Transport of charged vesicles through thin or thick epidermal samples with an applied electrical potential.
Inset gives the corresponding rate of penetration.

Example 58:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:
274 mg phosphatidylglycerol (DOPG)
226 mg Tween 80
^{3}H-DPPC (relative to DOPG)
Buffer as in previous examples
Vesicle/pore size ratio: 3.5

Electrical characteristics:

Electrical current: 0 mA, 0.2 mA
(current density:0, 0.048 mA cm^{-2})
Electrical potential: difference: 0.5 V

The barrier for this test was prepared by acting with trypsin for 7 hours on a heat-

separated murine epidermis sample. Instead of using fluorescent labels, radioactive phospholipids were used. Consequently, the samples were taken from receiver fluid manually and the readings were made with a beta-counter. Further difference between this and previous experiments was the use of murine, rather than human, epidermis and the relatively low electric current.

After a lag-time of approximately 30 min, constant vesicle transport was seen to commence. The rate of lipid transfer across the barrier, calculated from the following 90 min linear period, was approximately 4 microgramms per hour and cm^{-2}. Thereafter, no significant further transport was observed, in accordance with the findings from the other measurements with the non-radioactive labels.

This experiment clearly documents that charged carriers (here due to the presence of DOPG) can be used to electro-transport uncharged substances (here ^{3}H-DPPC) across a barrier with an applied electrical potential difference.

Figure 12:Electro-transport of uncharged molecules associated with charged, ultradeformable vesicles across murine epidermis in vitro.

CLAIMS

1. A preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability.

2. Preparation according to claim 1,
characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being selected to achieve, and in case, control said transport through the barrier under the influence of the electrical driving force.

3. Preparation according to claim 1,
characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force.

4. Preparation according to any one of claims 1 to 3, said penetrant being capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their permeation through the pores of the barrier subsequent to entry of said penetrant into said pores by means of an electrical driving force.

5. Preparation according to any one of claims 1 through 4, **characterized in that** the average diameter of the electrically charged penetrants or the charged association of penetrant and agent, is greater (by at least the factor of 2) than the average diameter of the pores of the barrier.

6. Preparation according to any one of claims 1 through 5, **characterized in that** the penetrant is formed by an electrically charged single molecule or an arrangement of electrically charged molecules and is associated with one or several charged or uncharged agent molecules.

7. Preparation according to any one of claims 1 through 5, **characterized in that** the penetrant is formed by an electrically neutral single molecule or an arrangement of electrically neutral molecules and is associated with at least one electrically charged agent, the quantity of electrical charges being sufficient to enable transport.

8. Preparation according to claim 6 or 7,

characterized in that the penetrants are suspended or dispersed in a liquid medium and comprise arrangements of molecules in the form of minute fluid droplets surrounded by a membrane-like coating of one or several layers of at least two kinds or forms of amphiphilic substances with a tendency to aggregate, said at least two substances differing by at least a factor of 10 in solubility in the, preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or the average diameter of hetero-aggregates comprising both said substances is smaller than the average diameter of homo-aggregates of the less soluble substance.

9. Preparation according to claim 8,

characterized in that the more soluble substance is the agent to be transported through the barrier, and has a propensity to form common larger structures with the less soluble substance.

10. Preparation according to claim 9,

characterized in that the common structure comprises a physical or chemical complex of the substances.

11. Preparation according to claim 8, 9 or 10,

characterized in that the more soluble substance tends to solubilize the penetrant droplet and the content of this substance is up to 99 mol% of the concentration required to solubilize the droplet, or else corresponds to up to 99 mol% of the saturating concentration in the unsolubilized droplet, whichever is higher.

12. Preparation according to claim 11,

characterized in that the content of the more soluble substance is below 50 %, especially below 40 % and most preferably below 30 %, of the respective solubilizing concentration of said substance.

13. Preparation according to claim 11,
characterized in that the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.

14. Preparation according to any one of claims 8 through 13,
characterized in that the less soluble self-aggregating substance is a lipid-like substance and the more soluble substance is a surfactant.

15. Preparation according to any one of claims 8 through 14,
characterized in that the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.

16. Preparation according to any one of claims 8 through 14,
characterized in that the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.

17. Preparation according to any one of claims 8 through 16,
characterized in that the average net surface charge density on a droplet is between 0.05 Cb m-2 (Coulomb per square meter) and 0.5 Cb m-2, preferably between 0.075 Cb m-2 and 0.4 Cb m-2, and particularly preferably between 0.10 Cb m-2 and 0.35 Cb m-2.

18. Preparation according to any one of claims 8 through 17,
characterized in that the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

19. Preparation according to any one of claims 8 through 17, **characterized in that** the weight amount of droplets in formulations for the use on human or animal mucosa is 0,0001 to 30 weight-%.

20. Preparation according to any one of claims 8 through 19, **characterized in that**, the agent is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and / or bronchospasmolyticum, an antibioticum, antidrepressivum and / or antipsychoticum, an antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an antihistaminicum, antihypertonicum, an antihypotonicum, anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardiotonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antiflue agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance such as an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter (agonist) for the activity of any of the above-mentioned agents or any combination of such agents.

21. Preparation according to any one of claims 8 through 20, **characterized in that** the liquid medium characteristics, especially the concentration and the composition of the supporting electrolyte, are selected so as to enable and / or control the rate or the efficiency of transport of the penetrant through the pores of the barrier.

22. Preparation according to any one of claims 8 through 21, **characterized in that** the supporting electrolyte, in particular a buffer, is selected among monovalent (1 : 1) or other low valency electrolytes, with the bulk concentration preferably below 150 mM, more preferably below 100 mM, even more preferably below 50 mM, and particularly preferably up to 10 mM.

23. A method for effecting the electrically driven transport of penetrants and associated molecules through the pores in a barrier, as defined in any one of the preceding claims, **characterized in that** a sufficient electrical potential is applied across the barrier.

24. Method according to claim 23, **characterized in that** the electrodes used to generate the electrical potential across the barrier are located on opposite sides or on the same side of the barrier and are arranged so as to ensure that most of the resulting electrical current will flow across the barrier.

25. Method according to claims 23 or 24, **characterized in that** the applied electrical potential value is chosen to be below 30 V, more often below 15 V, and even more preferably below 10 V, per cm2 of the barrier surface.

26. Method according to claims 23 or 24,
characterized in that the current driven across the barrier by the applied electrical potential is in the physiologically tolerable range, typically below 2 mA cm-2, preferably below 1 mA cm-2, more preferably below 0.6 mA cm-2 and most preferably up to 0.4 mA cm-2.

27. Method according to any one of claims 23 through 26,
characterized in that the electrode size is less than 200 cm2, more preferably below 100 cm2, especially below 50 cm2, most preferably below 10 cm2, or even below 5 cm2.

28. Method according to any one of claims 23 to 27,
characterized in that the electrically conductive material on or of the electrodes comprises at least one metal, in particular selected from precious metals, such as silver or palladium, and / or biocompatible salts or chemical complexes of such metals, preferably the biocompatible chlorides, and most preferably silver chloride.

29. Method according to any one of claims 23 to 28,
characterized in that at least one electrode compartment is loaded with electrically charged penetrants.

30. Method according to claim 29,
characterized in that the electrode is loaded at the application site or earlier.

31. Method according to claims 29 or 30,
characterized in that the electrode is loaded shortly before application, preferably within 360 minutes, more preferably within 60 minutes and even more preferably within 30 minutes.

32. Method according to claims 29, 30 or 31,
characterized in that the electrode is loaded with the electrically charged penetrant

pre-associated with molecules to be transported, in particular (biologically active) agents.

33. Method according to claims 29, 30 or 31,
characterized in that the electrode is loaded with the penetrant and the molecules to be transported, in particular agents that associate therewith during or after said loading.

34. Method according to any one of claims 23 through 33,
characterized in that one or more programmable, preferably small, hand-held or self-supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied electric potential.

35. Method according to any one of claims 23 through 34,
characterized in that different treatment areas are selected to control the transport.

36. Method according to any one of claims 23 through 35,
characterized in that the barrier is pre-treated, before initiating the electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

37. Method according to claim 36,
characterized in that the charged or uncharged penetrants used to pre-treat the barrier are similar or identical with those employed for the subsequent electrically driven transport.

38. Method according to claims 36 or 37,
characterized in that the charged or uncharged penetrants are non-occlusively applied for up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to

the initiation of electrically driven transport of charged penetrants and / or permeants across the barrier.

39. Method according to any one of claims 23 to 38,

characterized in that the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

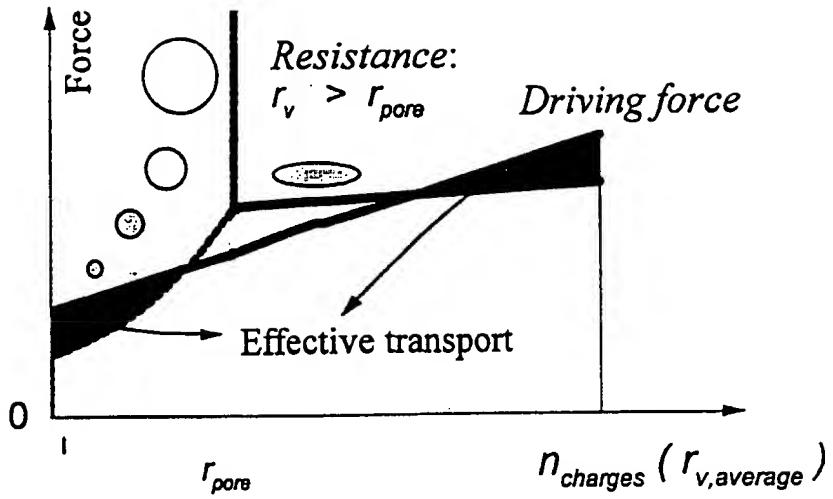
Force
Resistance:
$r_v > r_{pore}$
Driving force
Effective transport
0
r_{pore}
$n_{charges}$ ($r_{v,average}$)

Figure 1

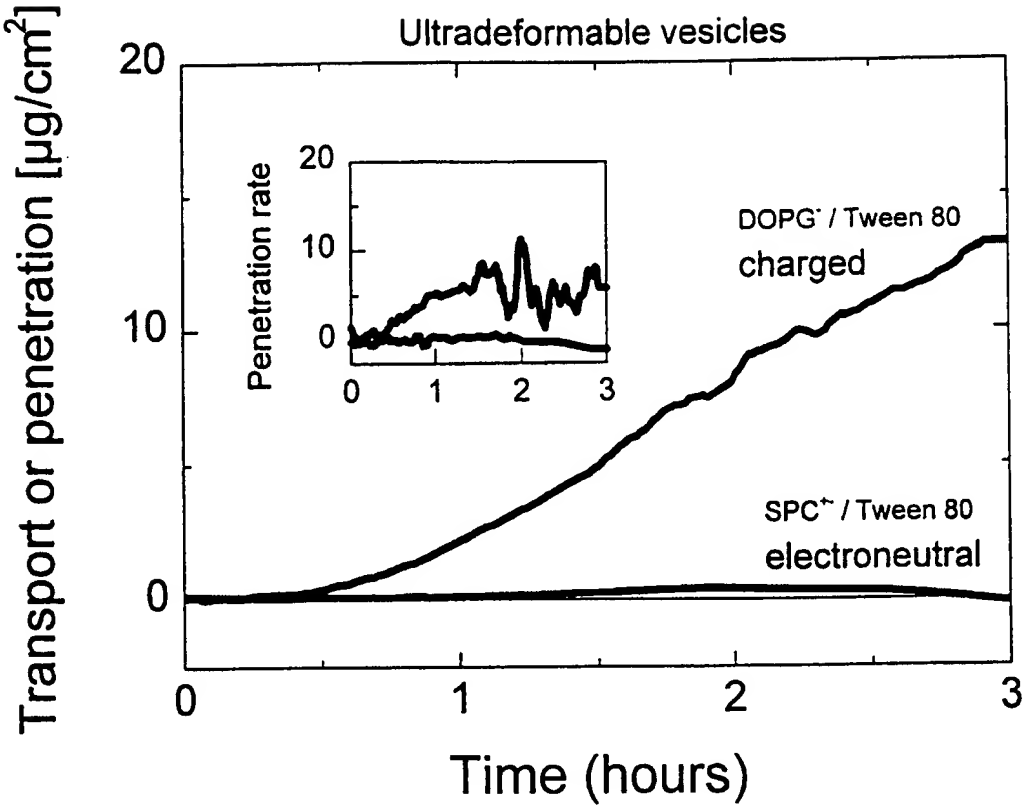
Ultradeformable vesicles
Transport or penetration [µg/cm²]
Time (hours)
Penetration rate
DOPG⁻ / Tween 80
charged
SPC⁺⁻ / Tween 80
electroneutral
0
10
20
0
1
2
3

Figure 2

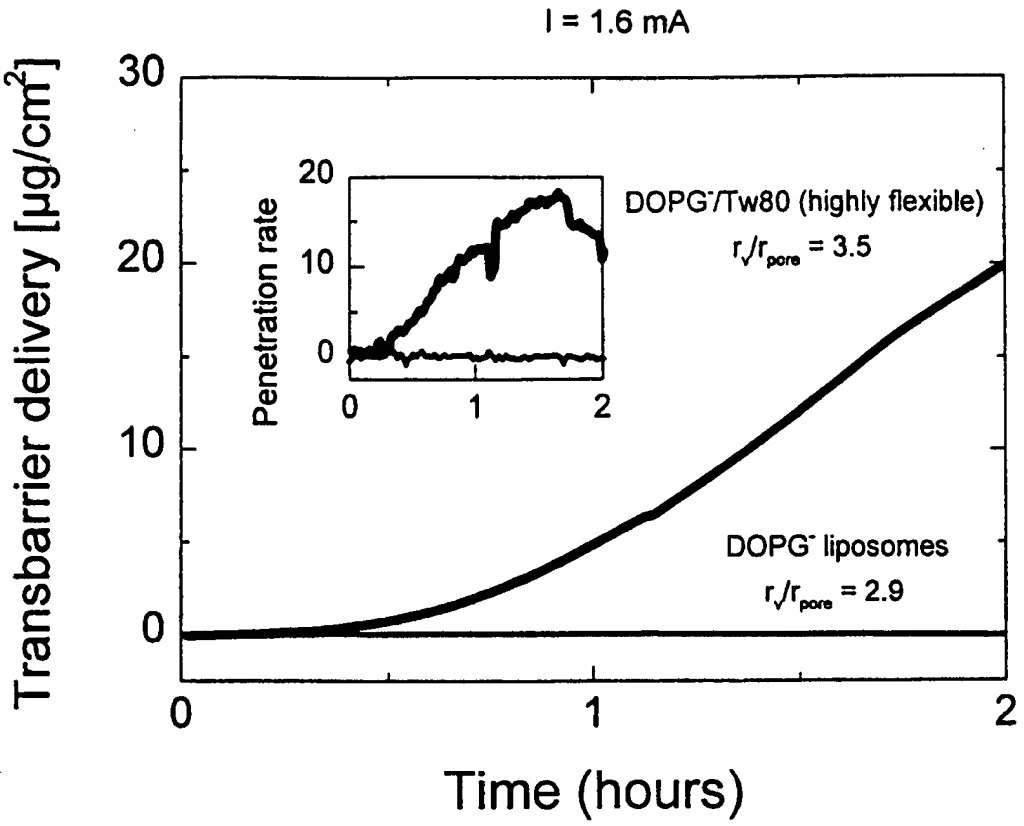
I = 1.6 mA
Transbarrier delivery [µg/cm²]
30
20
10
0
0
1
2
Time (hours)
Penetration rate
20
10
0
0
1
2
DOPG⁻/Tw80 (highly flexible)
r/r_pore = 3.5
DOPG⁻ liposomes
r/r_pore = 2.9

Figure 3

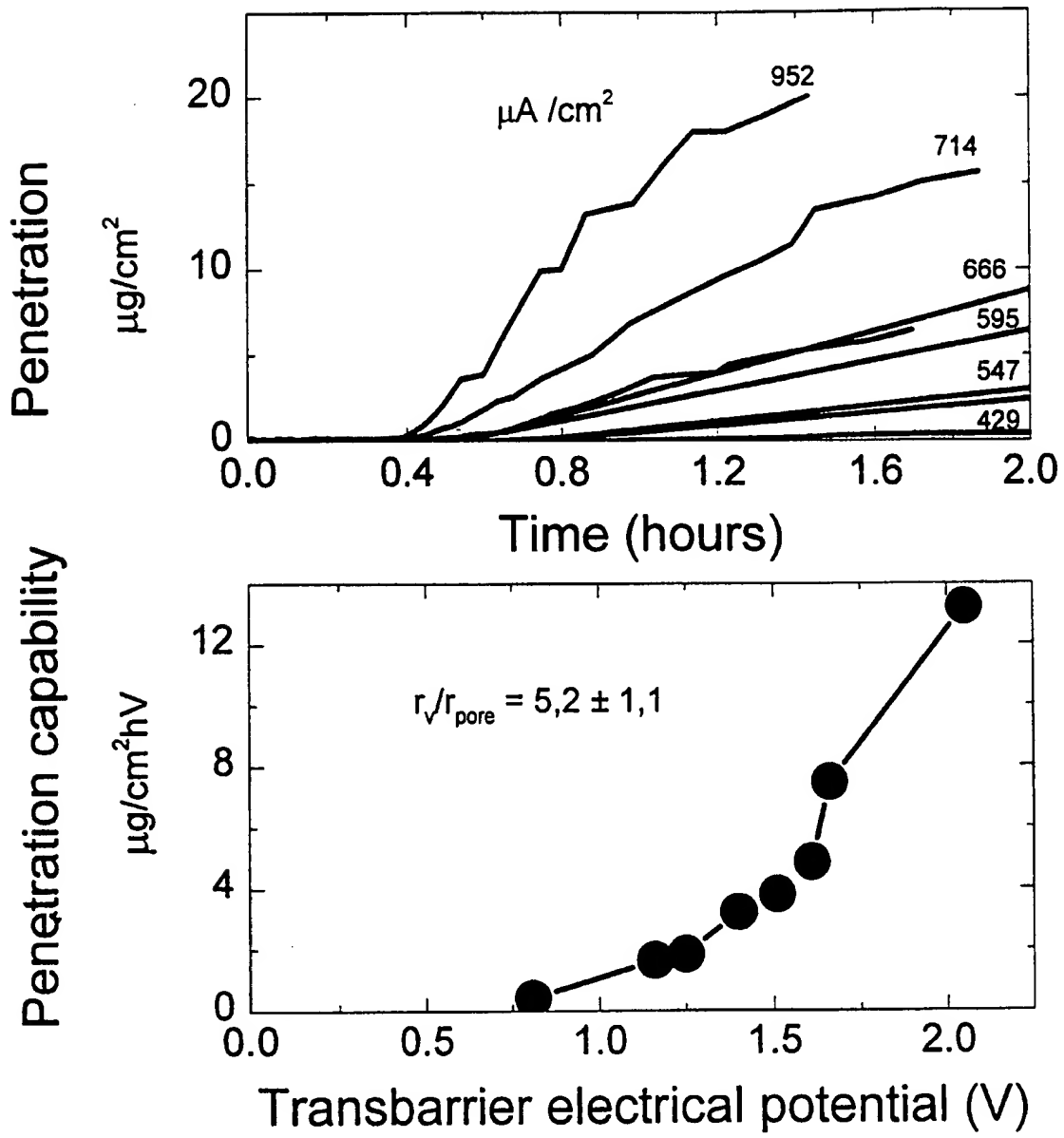
Penetration
µg/cm²
µA /cm²
952
714
666
595
547
429
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
Time (hours)
Penetration capability
µg/cm²hV
r_v/r_pore = 5,2 ± 1,1
12
8
4
0
0.0
0.5
1.0
1.5
2.0
Transbarrier electrical potential (V)

Figure 4

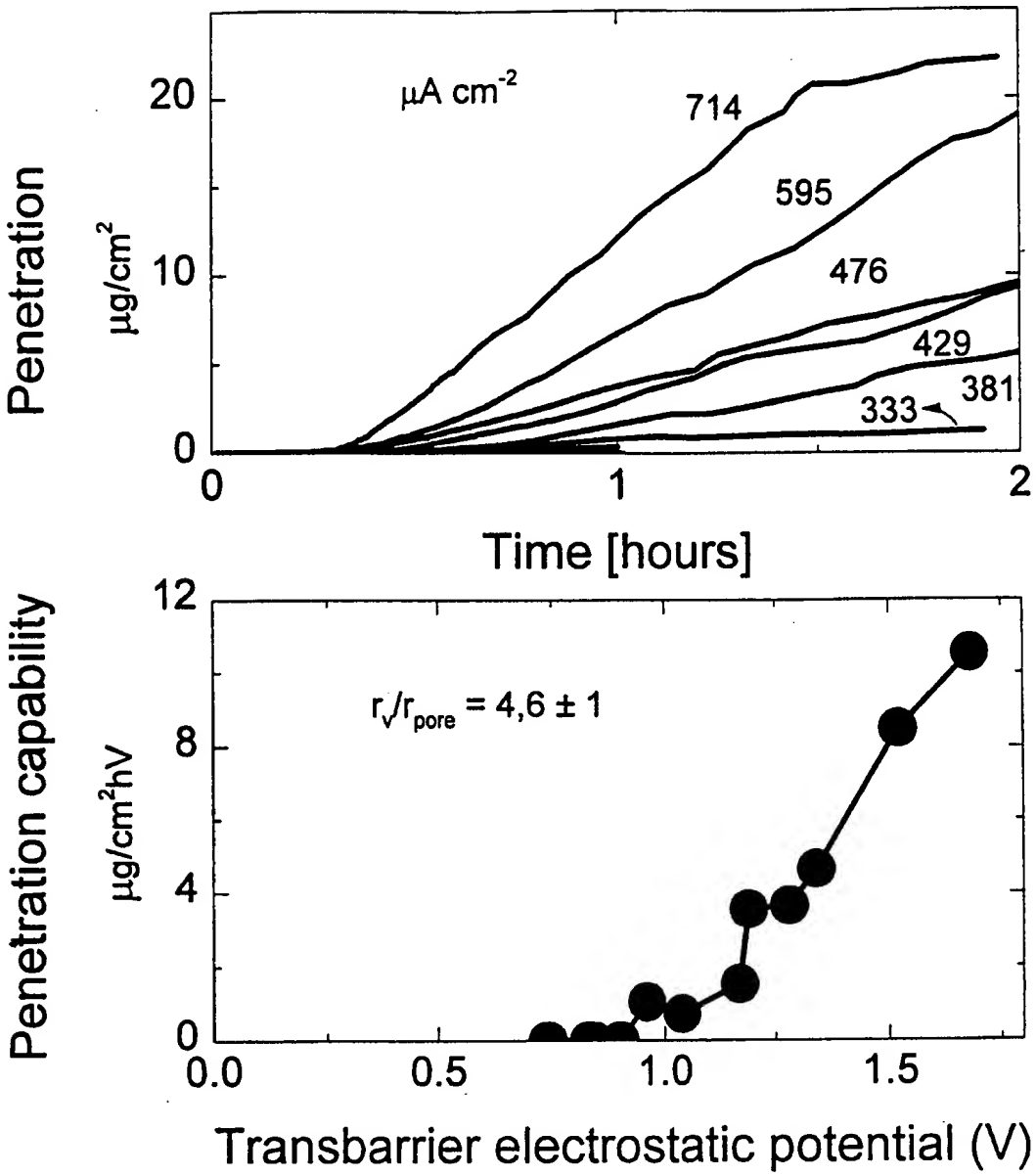
Penetration
μg/cm²
μA cm⁻²
714
595
476
429
381
333
20
10
0
0
1
2
Time [hours]
Penetration capability
μg/cm²hV
r√/r_pore = 4,6 ± 1
12
8
4
0
0.0
0.5
1.0
1.5
Transbarrier electrostatic potential (V)

Figure 5

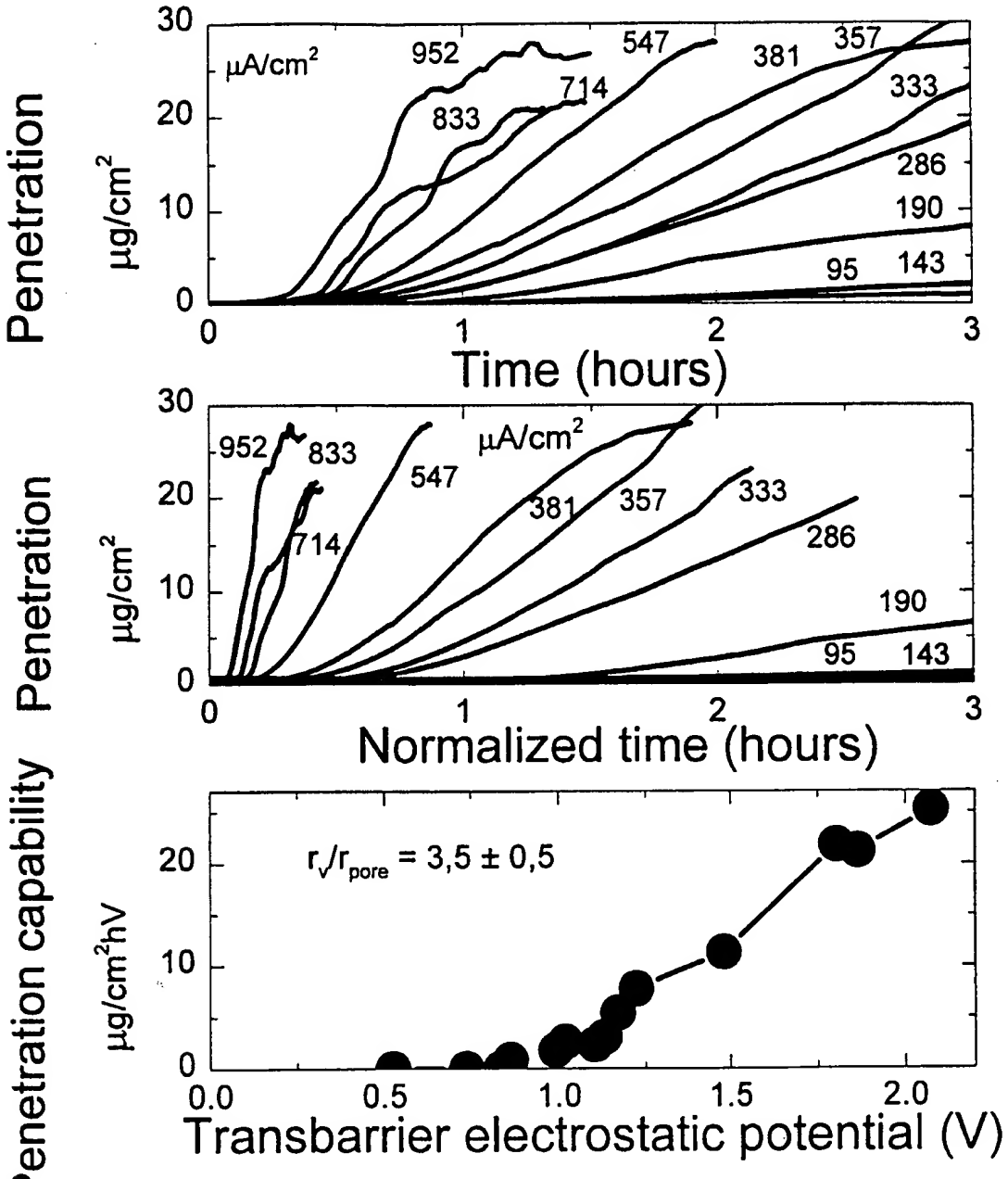
Penetration capability
Penetration
μg/cm²
μA/cm²
952
833
714
547
381
357
333
286
190
95
143
Time (hours)
Normalized time (hours)
μg/cm²hV
r_v/r_pore = 3,5 ± 0,5
Transbarrier electrostatic potential (V)

Figure 6

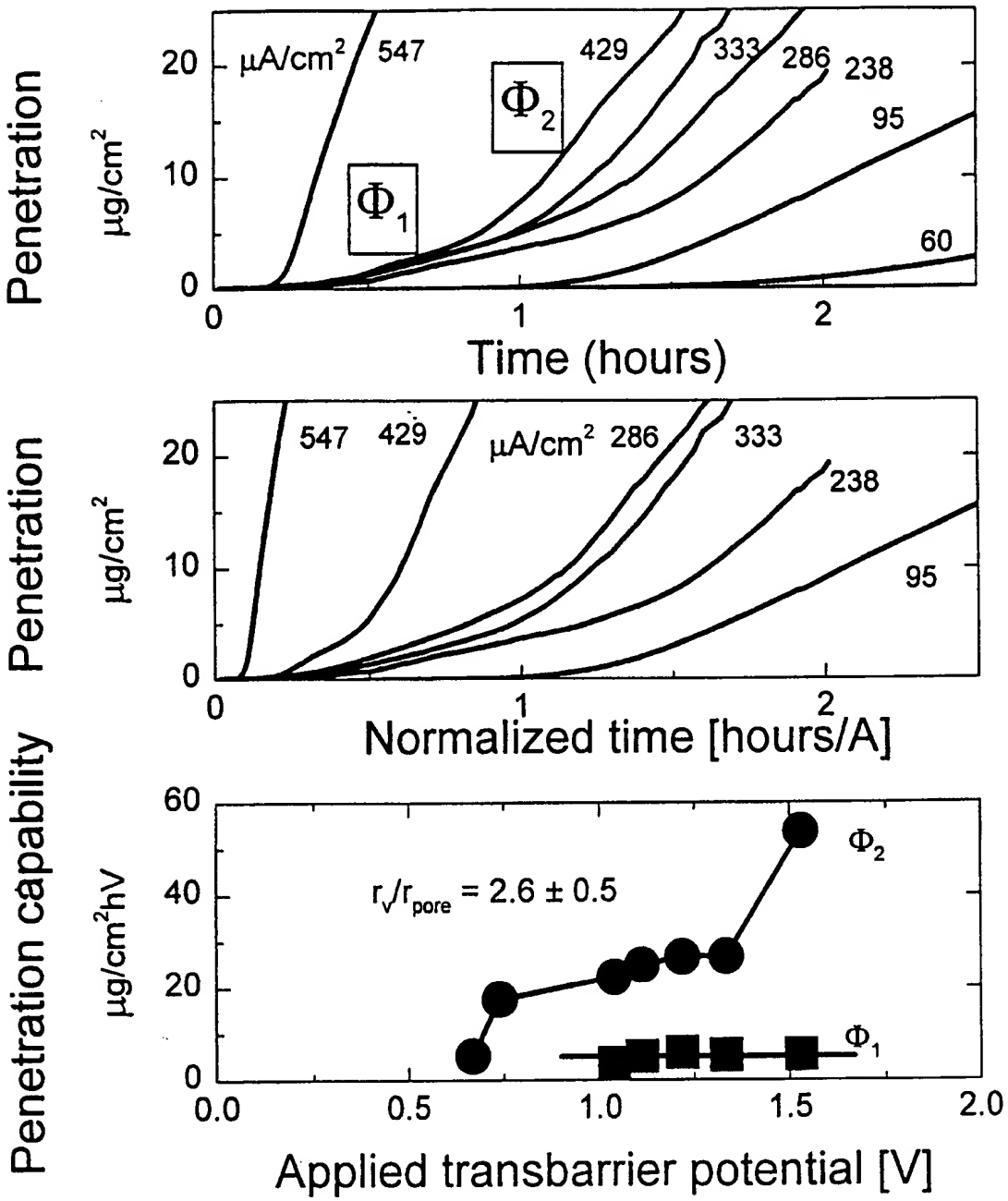
Penetration
μg/cm²
μA/cm² 547
429
333
286
238
95
60
Φ2
Φ1
Time (hours)
Penetration
μg/cm²
547
429
μA/cm² 286
333
238
95
Normalized time [hours/A]
Penetration capability
μg/cm²hV
rv/rpore = 2.6 ± 0.5
Φ2
Φ1
Applied transbarrier potential [V]

Figure 7

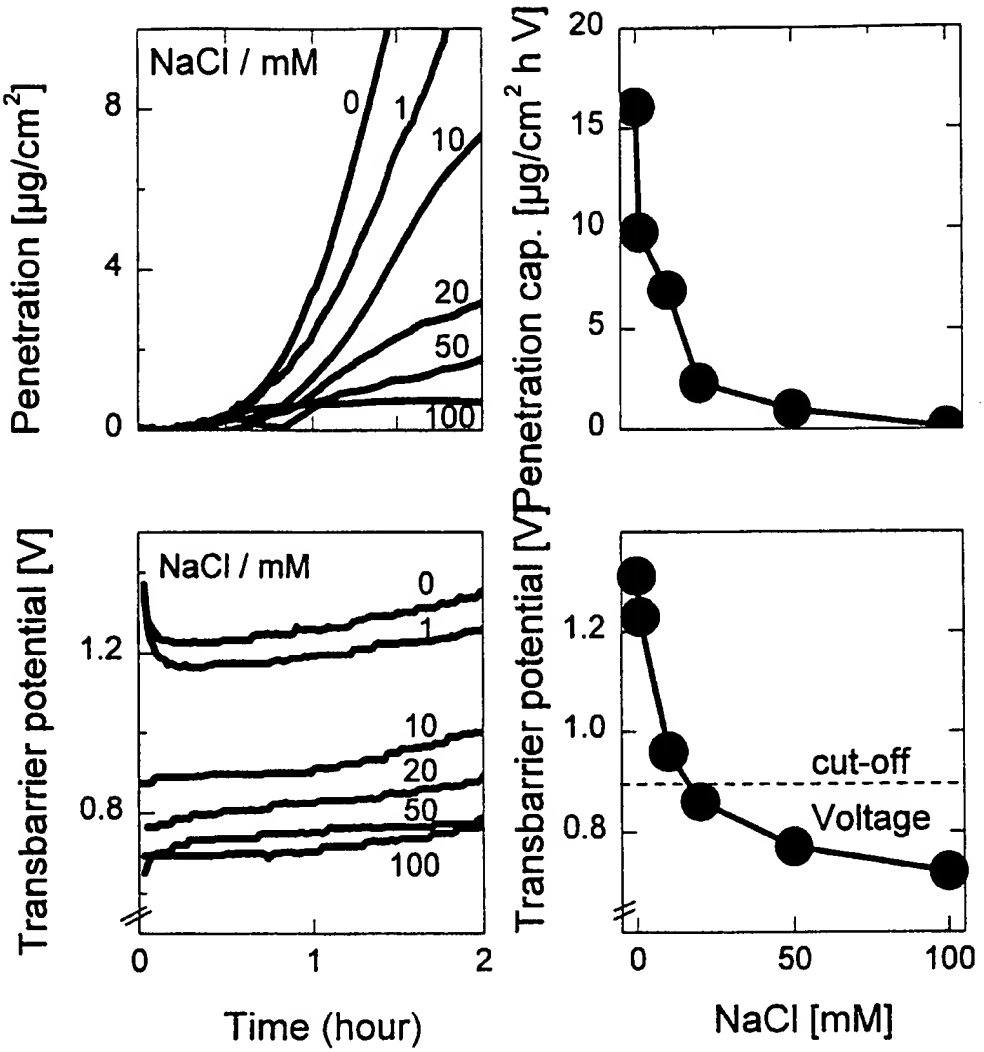
NaCl / mM
0
1
10
20
50
100
Penetration [µg/cm²]
Time (hour)
Transbarrier potential [V]
Penetration cap. [µg/cm² h V]
cut-off
Voltage
NaCl [mM]

Figure 8

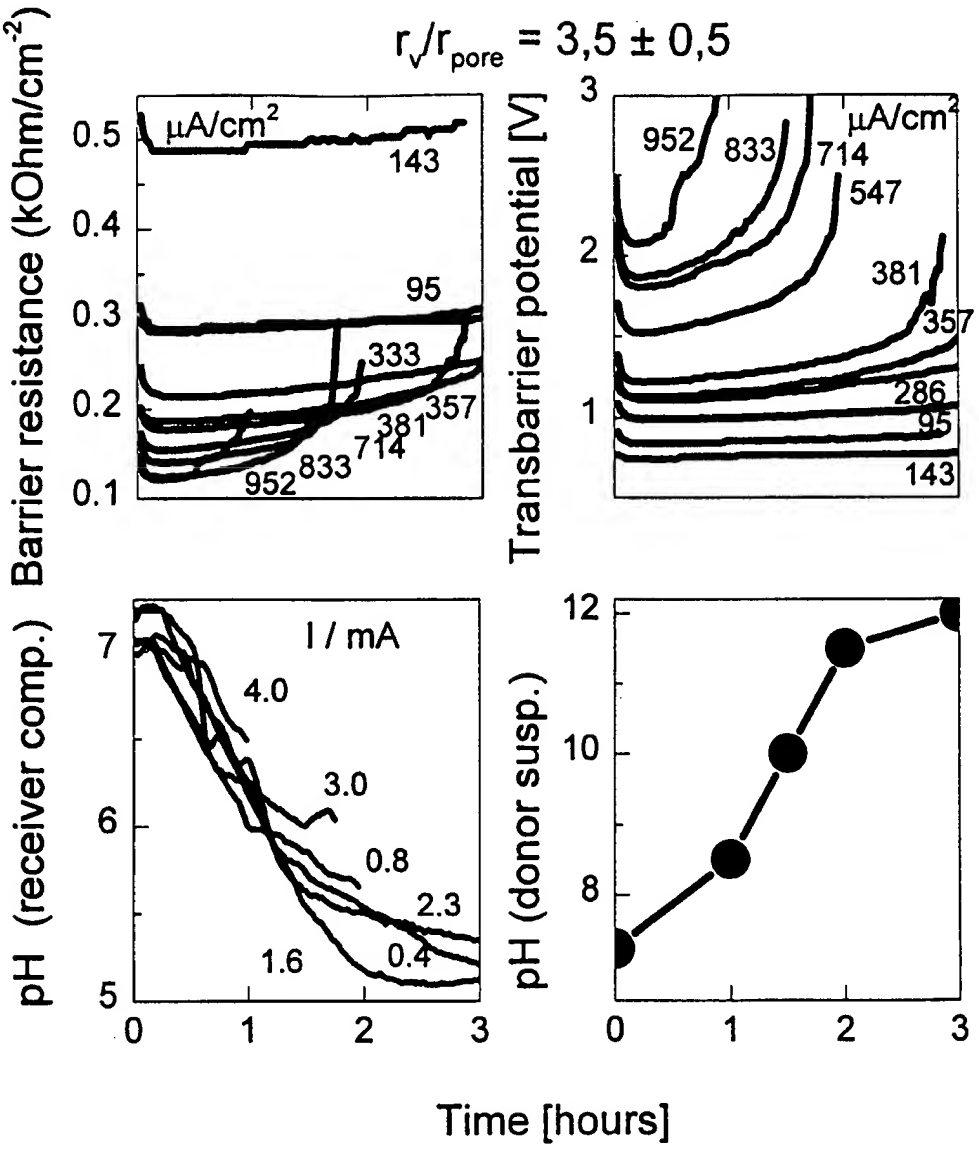
r_v/r_{pore} = 3,5 ± 0,5
Barrier resistance (kOhm/cm^{-2})
µA/cm^2
143
95
333
357
381
714
833
952
Transbarrier potential [V]
µA/cm^2
952
833
714
547
381
357
286
95
143
pH (receiver comp.)
I / mA
4.0
3.0
0.8
2.3
1.6
0.4
pH (donor susp.)
Time [hours]

Figure 9

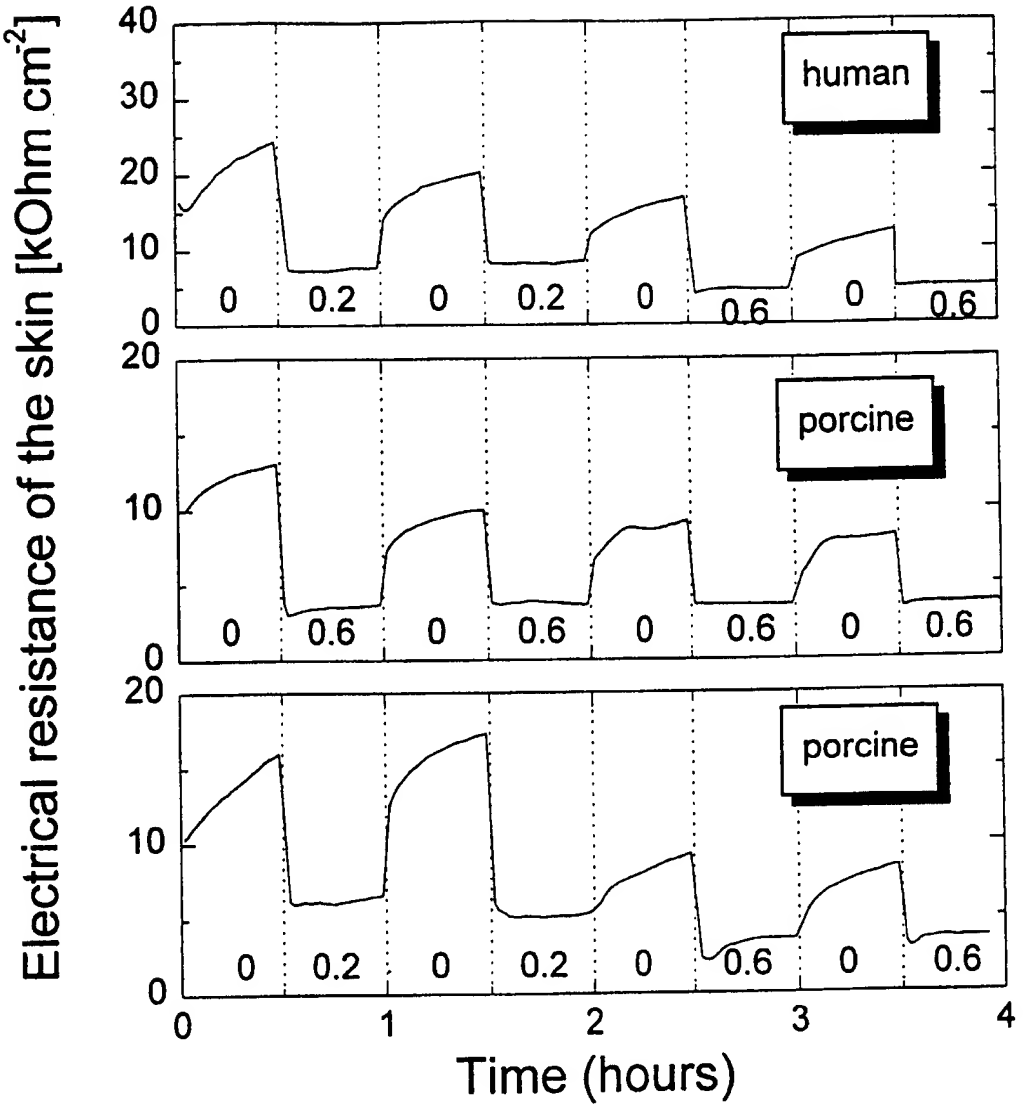
Electrical resistance of the skin [kOhm cm^{-2}]
human
porcine
porcine
0
0.2
0.6
Time (hours)

Figure 10

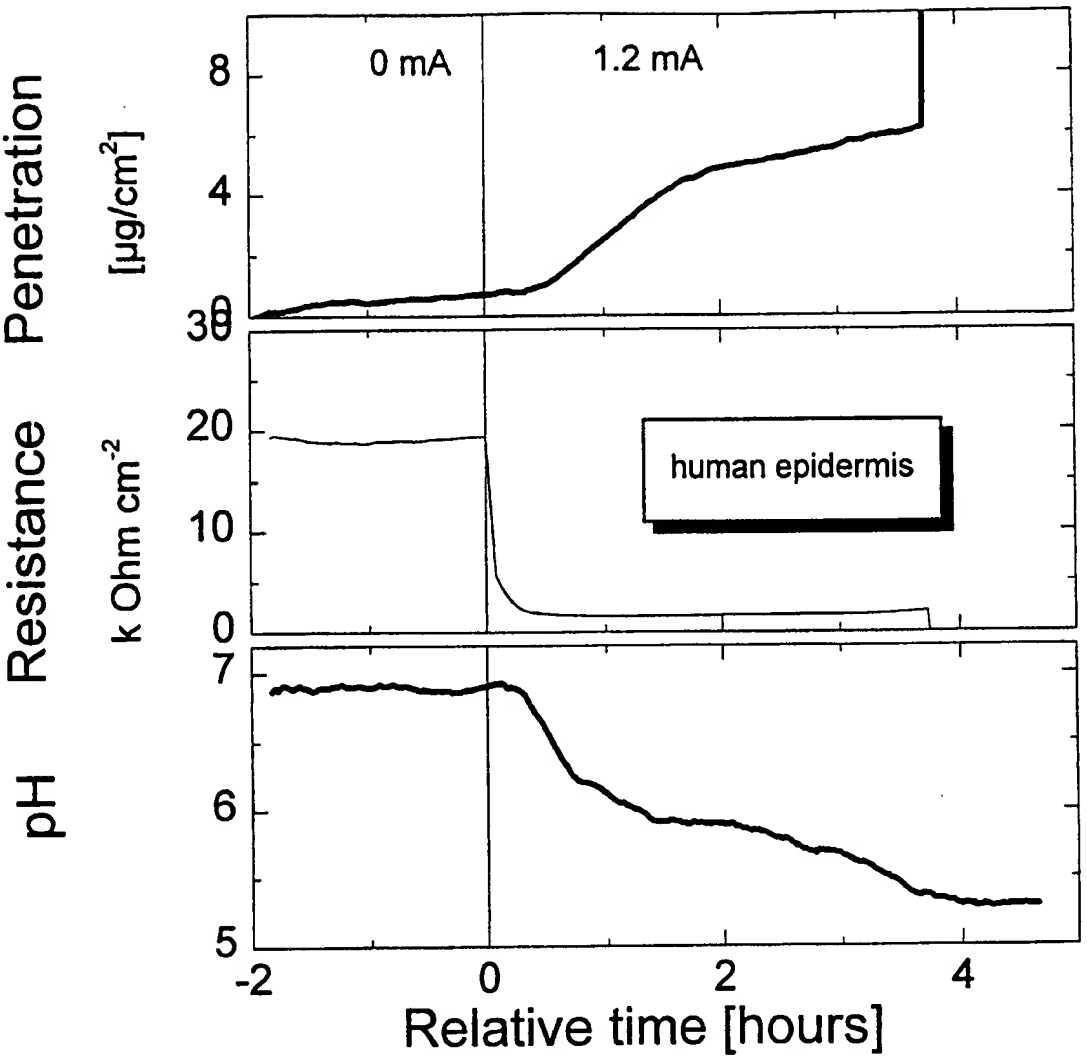
Penetration [µg/cm²]
Resistance k Ohm cm⁻²
pH
0 mA
1.2 mA
human epidermis
8
4
0
30
20
10
0
7
6
5
-2
0
2
4
Relative time [hours]

Figure 11A

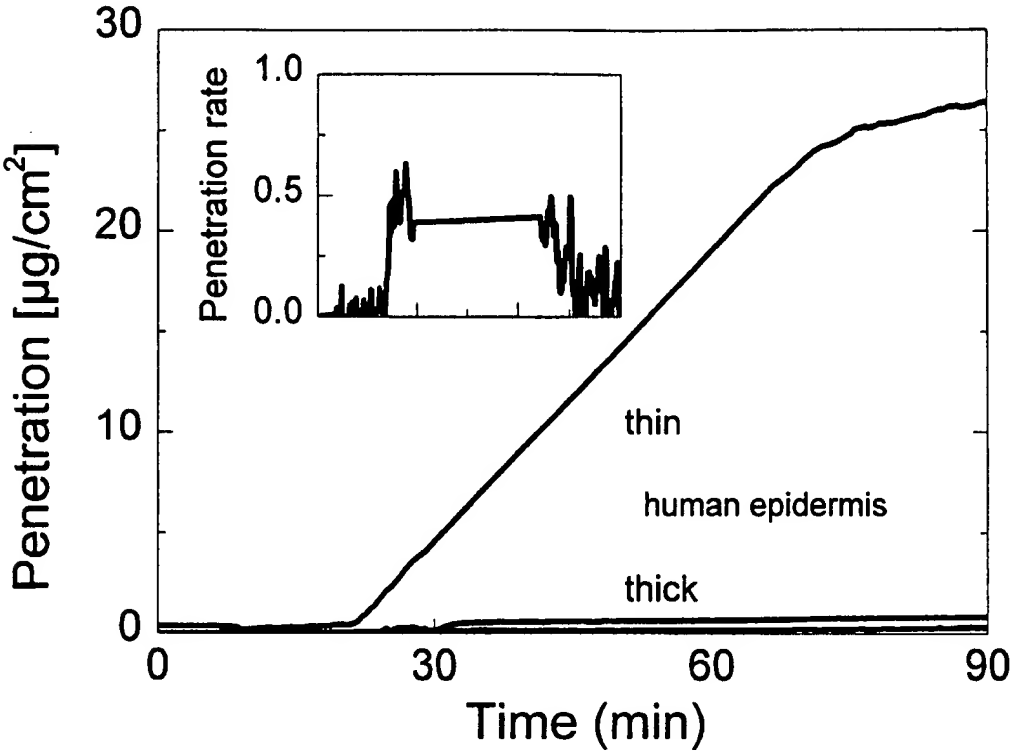
30
20
10
0
Penetration [µg/cm²]
0
30
60
90
Time (min)
Penetration rate
1.0
0.5
0.0
thin
human epidermis
thick

Figure 11B

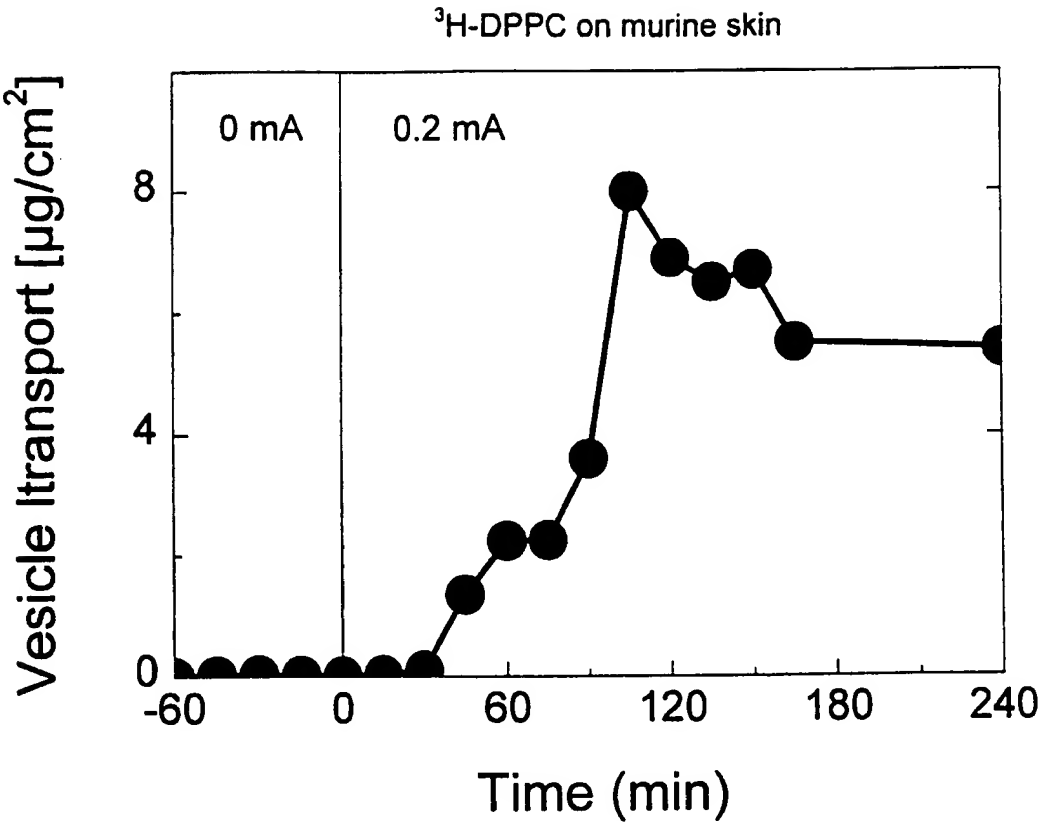
^{3}H-DPPC on murine skin
0 mA
0.2 mA
Vesicle ltransport [µg/cm^2]
8
4
0
-60
0
60
120
180
240
Time (min)

Figure 12

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 98/05539

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/00 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 552 666 A (KAO CORPORATION) 5 April 1985 see page 17; example 4 see claim 1 ---	1-22
A	EP 0 475 160 A (CEVC) 18 March 1992 cited in the application see page 37 - page 40; examples 32-49,62-98 --- -/--	1-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 April 1999	17/05/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

:national Application No
PCT/EP 98/05539

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	N.B. VUTLA ET AL.: "transdermal iontophoretic delivery of enkephalin formulated in liposomes" JOURNAL OF PHARMACEUTICAL SCIENCES , vol. 85, no. 1, January 1996, pages 5-8, XP000543850 Washington (US) cited in the application see the whole document ---	23-39
A	CHEMICAL ABSTRACTS, vol. 126, no. 8, 24 February 1997 Columbus, Ohio, US; abstract no. 108828k, S.B. KULKARNI ET AL.: "transdermal iontophoretic delivery of colchicine encapsulated in liposomes" page 1034; column 2; XP002101479 see abstract & DRUG DELIVERY, vol. 3, no. 4, 1996, pages 245-250, ---	23-39
A	WO 94 17792 A (AFFYMAX TECHNOLOGIES N.V.) 18 August 1994 see page 26; example 5 see claims 1,7-10,15-17 -----	23-39

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